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A COLLABORATIVE STUDY OF METHODS FOR THE DETERMINATION OF POTASSIUM, CALCIUM AND MAGNESIUM IN PLANT MATERIALS[1]

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[Received for publication February 4, 1960]

ABSTRACT

Thirteen Canadian laboratories collaborated in a 2-year study of methods of analysis of plant tissue. The investigation has shown that the flame photometric procedure for potassium and a modification of the chelatometric method using EDTA for calcium and magnesium give the most consistent and reproducible results. Further research is necessary before the flame photometer can be used for the reliable determination of calcium and magnesium.

INTRODUCTION

In the past few years the flame photometer has become a popular analytical tool in many branches of chemistry. A number of laboratories across Canada engaged in the analysis of plant tissues have adopted the new technique and purchased instruments of various types. The great saving in time that results from the application of flame photometry to routine chemical analysis has recommended the method to many workers, but some have found that the results are not always accurate. When it was learned that several people were experiencing the same difficulties, a collaborative study of methods of analysis was organized in the laboratories of the Department of Agriculture at Ottawa. The object of the study was to assess the accuracy of flame photometric analytical methods for the determination of potassium, calcium and magnesium in plant tissues of various types and to compare these methods for speed and accuracy with conventional chemical methods. The usual technique of employing standard samples was used. The following research officers participated: G. R. Webster, Experimental Farm, Saanichton, British Columbia; H. F. Fletcher, Experimental Farm, Agassiz, British Columbia; C. F. Bentley, University of Alberta, Edmonton, Alberta; J. A. Archibald, Ontario Department of Agriculture, Vineland, Ontario; L. S. Vickery, Tobacco Substation, Delhi, Ontario; G. M. Ward, Plant Research Institute, Ottawa, Ontario; H. B. Heeney, Plant Research Institute, Ottawa, Ontario; I. Hoffman, Analytical Chemistry Research Service, Ottawa, Ontario; J. E. Tasman, Pulp and Paper Research Institute, Pointe Claire, Quebec; J. Tardif, Quebec Department of Agriculture, Quebec City, Quebec; D. C. MacKay, Research Station, Kentville, Nova Scotia; J. J. Jasmin, Horticultural Organic Soil Substation, Ste. Clothilde de Chateauguay, Quebec; J. L. Mason, Research Station, Summerland, British Columbia.

[1]Scientific Contribution No. 52, Plant Research Institute, Research Branch, Canada Department of Agriculture, Ottawa, Ont.

PROCEDURE

The investigation extended over a period of 2 years, 1956-1958, and was divided into two distinct phases, Part 1 and Part 2. In Part 1 each collaborator was asked to analyse standard samples by procedures currently in use in his own laboratory. The standard samples consisted of the following: apple leaves, pear leaves, peach leaves, grape petioles and tomato leaves. These were collected, dried, ground to a fine powder and thoroughly mixed. Two lots of each in sealed containers were sent to all collaborators. The samples were ashed in duplicate and two determinations made on each extract. In order to eliminate one of the variable factors, specific instructions were given for the determination of the moisture content of the air-dried samples.

The collaborating laboratories were equipped with a great variety of flame photometers and this has been taken into account in assessing the results. Following is a list of the various instruments used:

Beckman DU with hydrogen and oxygen
Beckman DU with acetylene and oxygen
Beckman DU with acetylene and oxygen and old type burner
Beckman Model B with acetylene and oxygen
Perkin-Elmer with acetylene and oxygen and internal standards
E.E.L. with propane gas and compressed air.

In Part 2, single samples of the following tissues were sent to each collaborator: strawberry leaves, raspberry leaves, peach leaves, apple leaves, tomato leaves, corn leaves, grape petioles, brome grass plants, and oat grain.

Because we suspected that one source of error might be the variation within and between laboratories in the procedures for ashing and preparing extracts prior to analysis, an additional set of samples, consisting of an acid extract of the ash of each of the dried samples, was prepared and sent to collaborators in the second year.

In Part 2 of the investigation, flame photometer procedures were outlined for the use of collaborators and a compensating solution was described for use with standards. One function of this solution was to provide a background of emission spectra roughly similar to what might be found in the average plant extract. It contained 150 p.p.m. potassium, 150 p.p.m. calcium, 75 p.p.m. magnesium, 10 p.p.m. sodium, 13.5 p.p.m. phosphorus. In each determination the particular element being measured was omitted from the compensating solution added to the standard.

It is a well known fact that phosphorus causes a positive interference in the determination of calcium by the flame photometer. Two methods of eliminating this effect have been suggested. The first consists of flooding both standard and sample with relatively large amounts of phosphate, i.e. 2000 p.p.m. The second method is the removal of phosphate from the sample by precipitation with an iron salt in alkaline solution. Both procedures were recommended to collaborators but only two attempted the removal procedure.

The chemical methods used in this study by some of the collaborators were colorimetric or turbidimetric procedures described by Ward and Johnston (3). In Part 2 additional procedures were included for deter-

mining calcium and magnesium by titration with ethylene diamine tetra acetic acid (EDTA) (1, 2) as modified by Ward and Johnston (3).

RESULTS

The data received from all collaborators in this lengthy investigation have been tabulated and analysed. The results of the statistical analysis have been summarized and are presented in the accompanying Tables 1-4 with sufficient data to illustrate our findings. The plant materials used in this study were selected to be representative of a reasonably broad range of concentrations of the particular elements being considered. Since it is impossible to illustrate this point in the summarized data, it should be noted here that potassium concentrations varied from 0.30 to 3.42 per cent of dry weight, calcium from 0.07 to 2.88 per cent, and magnesium from 0.08 to 0.62 per cent. The data did not reflect any special difficulties in the analysis of any single type of tissue.

In Part 1 (Table 1) the value given for each element represents the average of eight determinations on each of the five samples. The data obtained by different methods have not been separated in Table 1. The mean for all laboratories has been calculated, also the standard error of the mean, expressed as a percentage value. This final value gives a measure of accuracy of the methods in the hands of various investigators over the range of samples used. The method for phosphorus was not being studied in this investigation, but phosphorus determinations on all samples were requested and were used as a means of testing the accuracy of the technique of sample preparation in each laboratory. With a mean value for phosphorus content of 0.239 per cent of the dry weight the standard error amounted to only 1.7 per cent. This represents excellent agreement. The percentage standard error of the mean for potassium was 2.9 which indicated that reasonably good agreement between laboratories was obtained for this

TABLE 1. — SUMMARY OF ANALYTICAL RESULTS FROM ALL METHODS
Part 1. 1956-1957
(expressed as per cent of dry weight)

Collaborator	Potassium	Calcium	Magnesium	Phosphorus*
1	1.68	2.35	.421	.227
2	1.59	1.21		
3	1.54	2.25	.244	
4	2.30	1.52*	.418*	.232
5		1.51*		
6	1.48*	1.49*	.510*	.246
7	1.66	1.42*	.457*	.242
8		1.39		
9	1.70	1.51	.438	.242
10	1.73	1.63*	.422*	.246
11	1.74*			
12	1.62	1.27	.365	.244
13	1.63	1.16		
Mean	1.70	1.56	.409	.239
Standard error of the mean	0.0499	0.101	.083	.0041
% Standard error of mean	2.9	6.5	20.3	1.7

*Non-flame photometer methods (3)

TABLE 2. — SUMMARY OF ANALYTICAL RESULTS FOR POTASSIUM
Part 2. 1957-1958
(expressed as per cent of dry weight of tissue or p.p.m. of extract)

Collaborator	Photometric		Turbidimetric	
	Tissue	Extract	Tissue	Extract
1	1.87	133		
2	1.70	120	1.70	120
3	1.71	137		
4	1.90	145	1.98	157
5	1.82	129		
6	1.88	136	1.93	136
7	1.82	134		
8	1.72	120		
9	1.39	136		
10		135		
Mean	1.75	132	1.87	138
Standard error of the mean	0.061	2.5	0.127	5.1
% Standard error	3.5	1.9	6.8	3.7

TABLE 3. — SUMMARY OF ANALYTICAL RESULTS FOR CALCIUM
Part 2. 1957-1958
(expressed as per cent of dry weight of tissue or p.p.m. of extract)

Collaborator	Photometric		Turbidimetric		EDTA 1		EDTA 2	
	Tissue	Extract	Tissue	Extract	Tissue	Extract	Tissue	Extract
1								
2			1.40	67	1.28	84	1.24	82
3	1.32		1.26		1.24	82	1.27	86
4	1.59				1.32			
5	1.34	97						
6	1.26		1.16	77			1.23	81
7		84					1.22	78
8			1.21	88	0.89	72	1.22	81
9	1.31	82	1.25	92	1.26	81	1.25	80
10	1.55	85						
11	1.09	74						
12					1.17	75	1.22	80
Mean	1.35	84	1.26	81	1.19	79	1.24	81
Standard error of the mean	0.103	7.3	0.063	6.2	0.112	4.4	0.015	1.3
% Standard error	7.6	8.7	5.0	7.7	9.4	5.6	1.2	1.6

analysis. However, the percentages for calcium and magnesium indicated that the results did not fall within acceptable limits of error. This emphasized the necessity for proceeding with Part 2 of the study.

The data for Part 2 are shown in Tables 2, 3 and 4 where each figure in the "Tissue" columns is the average of single analyses on duplicate ashings on each of nine samples. Each figure in the "Extract" columns is the average of duplicate analyses of the prepared extracts of nine samples. The data for each analytical method are shown separately. In Table 2 the percentage standard error of the mean for the flame photometer procedure

TABLE 4. — SUMMARY OF ANALYTICAL RESULTS FOR MAGNESIUM
Part 2. 1957-1958
(expressed as per cent of dry weight of tissue or p.p.m. of extract)

Collaborator	Photometric		Colorimetric		EDTA 1		EDTA 2	
	Tissue	Extract	Tissue	Extract	Tissue	Extract	Tissue	Extract
1					.316	29.2		
2	.294		.442	16.4	.304	22.1	.311	22.4
3	.284	19.6	.278		.331	23.3	.333	22.4
4	.272	21.1						
5							.321	23.6
6			.328	24.4			.316	23.0
7	.322	25.4	.288	21.9	.279	15.8	.310	24.2
8	.307	22.4	.310	22.4	.311	23.3	.315	23.9
9	.274	21.0						
10	.261	21.0						
11					.313	25.0	.300	23.6
Mean	.288	21.8	.329	21.3	.309	23.1	.315	23.3
Standard error of mean	.022	1.47	.034	2.09	.021	2.24	.014	0.78
% Standard error	7.6	6.8	10.3	9.8	6.8	9.7	4.5	3.4

for potassium in dried samples is 3.5 which is an acceptable degree of precision. It is apparent that, although one collaborator was out of line, reliable analytical results may be obtained throughout a wide variety of flame photometer conditions and that reported data may be accepted with confidence. The data from the turbidimetric procedure show a standard error of 6.8 per cent. Although no valid statistical comparison of the two methods is possible, because of lack of sufficient data for the turbidimetric procedure, it may be said that the wide variation in results makes this method unacceptable for precise analysis.

The percentage standard error calculated from the analytical data for the prepared extract was lower in both instances than that for the analysis of dried tissue. From this it would appear that more consistent results were obtained in the analysis of the prepared extracts than in the analysis of the dried samples. Inspection of the data reveals, however, that this difference depends mainly on the aberrant results of one collaborator in the analysis of the dried tissue. In spite of the excellent results obtained with the phosphorus test in Part 1, it is evident that a certain margin of error may be introduced during ashing and preparation of extracts from dried samples. In most instances, however, the difference is so small that it has very little effect on the acceptable accuracy of the final analytical results.

In Table 3 four methods for determining calcium are compared. The flame photometer method did not produce acceptable results. The turbidimetric method also lacked precision. The first EDTA procedure failed to give good results, but the second EDTA method had a percentage standard error of 1.2 for dried samples and 1.6 for extracts. The per cent standard error for the analysis of extracts by the flame photometer and by the turbidimetric procedure was higher in each case than that for the analysis

of tissue. This apparent discrepancy simply reflects the general lack of precision of these two methods of analysis.

The conclusions drawn from the analysis of magnesium by four methods (Table 4) are similar to those described for calcium. The percentage standard error values for the flame photometer method, the colorimetric method and the first EDTA method were all beyond the acceptable limits, while the corresponding values for the second EDTA method were both less than 5 per cent.

DISCUSSION

The information accumulated in this investigation and summarized in the foregoing sections has clarified a number of points about the use of the flame photometer. The reliability of this technique for the determination of potassium in plant tissues has been confirmed. Precise results can be obtained with many types of instrument which may vary widely in their conditions of analysis, e.g. flame temperature.

The flame photometric method for measuring calcium and magnesium has not yielded satisfactory results in this study. The variation in results between laboratories is much greater than can be accepted as normal experimental error. The removal of phosphate or the compensation for its interference has not given the necessary accuracy to the method. Based on comparison with the more acceptable EDTA method 2, it appears that the flame photometer method gives results that are too high for calcium and too low for magnesium. Further research is necessary on the flame photometer procedure before consistent and acceptable analytical results can be obtained.

The three chemical procedures that were used for purposes of comparison in this study have proved to be somewhat unreliable in the hands of various investigators. All three methods were designed to be used as "rapid tests" at the expense of a certain measure of accuracy and it has long been recognized that they are subject to some error. The turbidimetric sodium potassium cobaltinitrite procedure for potassium is unsatisfactory for precise comparisons because the composition of the precipitate may vary with environmental conditions. The turbidimetric procedure for calcium which depends upon the formation of a calcium soap of two fatty acids is a rapid technique but subject to considerable variation. The colorimetric thiazole yellow method for magnesium may give variable results depending on the source of the dye used to produce the colour. The analytical results obtained in this study are a fair reflection of the measure of accuracy that can be expected from these semi-precise methods.

The results obtained in this investigation have demonstrated that the modified EDTA method for the determination of calcium and magnesium is rapid, accurate and reproducible. Although the principle is the same in both EDTA procedures, the second method is easier to manipulate and has given more precise results. The procedure involves the reading of a rather difficult titrimetric end-point, but with a little practice all collaborators easily overcame this difficulty.

On the basis of these studies the authors recommend the flame photometric procedure for the determination of potassium in plant tissue and the

EDTA titration method using eriochrome black T and cal red for the determination of calcium and magnesium. The general use of these methods should increase the reliability of reported data on plant analysis in agricultural experiments.

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A STUDY OF THE EFFECTS OF TEMPERATURE AND OTHER FACTORS UPON THE GERMINATION OF VEGETABLE CROPS

III. VEGETABLE VINE CROPS[1]

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[Received for publication March 23, 1960]

ABSTRACT

A study was made of the effects of temperature and duration of exposure upon the germination of cucumber, muskmelon, and watermelon seed. Seeds of four varieties of each crop were germinated at controlled temperatures by rolled-towel method. Muskmelon seed germination was significantly reduced as temperature decreased from 60°F. through 55°, 50° and 45°F. On the other hand, germination of cucumber and watermelon seed was not significantly altered by temperature. Prolonging the duration of exposure to 15 or 22 days resulted in significant reductions in the germination of watermelons but not of the other crops. The interaction of temperature with duration of exposure caused significant differences in the germination of the three crops. At 45° and 50°F., germination decreased generally as exposure lengthened to 15 days and then increased sharply at 22 days. It is postulated that this increase was the result of temperature conditioning or some similar reaction. At 55° and 60°F., germination increased slightly as exposure increased to 8 or 15 days, followed by a decrease at 22 days. This effect was most pronounced at 60°F., probably due to greater fungal activity at that temperature.

It is concluded that satisfactory germination of the vine crops can be obtained after relatively long exposure to temperatures lower than the previously accepted minima.

INTRODUCTION

The vegetable vine crops are potentially important crops in the irrigated areas of southern Alberta. Cucumbers, pumpkins, and squash have been grown for processing for many years, while muskmelons and watermelons have been grown successfully on a limited scale. One of the problems associated with the production of these crops, particularly cucumbers, muskmelons and watermelons, has been the difficulty of obtaining satisfactory stands. This problem appears to be associated with cold soil conditions, as poor stands are most prevalent in cool seasons.

Literature dealing with the germination of vine crops is limited and only brief references are made in general horticultural textbooks (4, 5, 7). The consensus is that cucumbers, muskmelons, and watermelons, as a group, are warm-season crops and do not germinate or develop properly under conditions of low soil temperature. Jones and Rosa (4) and Shoemaker (7) state that cucumbers and muskmelons will not germinate below 50°F., while Kotowski (6) found that the lower limit of germination for these two crops was between 11° and 18°C. (51.8° and 64.4°F.). These authors do not mention the lower limit for germination of watermelons, but Knott (5) states: "Germination of watermelon seed is slow and poor at soil temperatures below 70°F." Official seed germination tests, as specified by the United States Department of Agriculture, require the exposure of vine crop seed to temperatures alternating from 20° to 30°C. (68° to 86°F.)

[1]Contribution from the Horticulture Section.

TABLE 1. — MEAN GERMINATION OF CUCUMBERS (IN DEGREES) AS AFFECTED BY (A) VARIETY, (B) TEMPERATURE, AND (C) DURATION OF EXPOSURE

A		B		C	
Variety	Mean germination	Temperature	Mean germination	Days exposed	Mean germination
		°F.			
National Pickling	72.1 (90.6)[2]	45	69.7 (88.0)	1	71.9 (90.3)
Ohio MR17	72.0 (90.5)	50	70.6 (89.0)	8	72.8 (91.3)
Straight 8	70.9 (89.3)	55	69.8 (88.1)	15	68.9 (87.0)
Marketer	67.6[1] (85.5)	60	72.4 (90.9)	22	68.9 (87.0)
L.S.D. (0.05)	±2.2	L.S.D.	n.s.	L.S.D.	n.s.

[1]Differs significantly from the germination of National Pickling
[2]Per cent germination in parentheses

TABLE 2. — MEAN GERMINATION OF MUSKMELONS (IN DEGREES) AS AFFECTED BY (A) VARIETY, (B) TEMPERATURE, AND (C) DURATION OF EXPOSURE

A		B		C	
Variety	Mean germination	Temperature	Mean germination	Days exposed	Mean germination
		°F.			
Farnorth	61.9 (77.8)[3]	45	63.1[2] (79.6)	1	66.1 (83.6)
Cornell Delicious	61.7 (77.5)	50	63.6[2] (80.2)	8	66.8 (84.5)
Sugar Rock	69.5[1] (87.7)	55	66.7[2] (84.4)	15	64.1 (80.9)
Hales Best	70.5[1] (88.9)	60	70.2 (88.5)	22	66.5 (84.1)
L.S.D. (0.05)	±1.5	L.S.D. (0.05)	±1.4	L.S.D.	n.s.

[1]Differs significantly from germination of Farnorth
[2]Differs significantly from germination at 60°F.
[3]Per cent germination in parentheses

for periods up to 7 days for cucumber, 10 days for muskmelon, and 14 days for watermelon (8). Studies by other seed technologists (1, 2) confirm the use of this temperature range for germination tests. However, no reference was made to the lower temperatures at which the vine crops would germinate.

This study is not concerned solely with the inability of the seed to germinate at continuous low temperatures, as reported by Kotowski (6), but also with the ability of the seed to germinate after exposure to low temperatures for varying periods of time.

MATERIALS AND METHODS

The following variables were considered:

1. *Temperature.*—Controlled temperature chambers were maintained at 45°, 50°, 55°, and 60°F. throughout the study.

2. *Duration of exposure.*—The seed was exposed to each of the above temperatures for periods of 1, 8, 15, and 22 days.

3. *Crops and varieties.*—The following kinds and varieties were subjected to all combinations of temperature and duration of exposure:

Cucumber — National Pickling, Straight 8, Marketer, and Ohio MR17
Muskmelon — Farnorth, Hales Best, Sugar Rock, and Cornell Delicious
Watermelon — New Hampshire Midget, Early Canada, Sweet Sensation, and Sweet Siberian.

The first variety, in each group, was the standard.

4. *Sampling.*—All treatments were sampled four times.

The rolled-towel seed tester technique (3, 8) was used. This is a departure from previous trials, in which soil and vermiculite (9) and vermiculite alone (10) were used. The paper towels were moistened with water to which a fungicide (Fermate) had been added to reduce the incidence of fungal infection.

Each crop, i.e., cucumber, muskmelon or watermelon, constituted a separate trial. Each trial contained four plots representing the four temperatures. Each plot consisted of 16 randomly arranged sub-plots, representing all combinations of the four varieties and the four durations of exposure. Each sub-plot was a rolled-towel containing 100 seeds. Observations were made on four samples of each sub-plot.

After seeding, the rolled-towels were enclosed in plastic bags and placed in the controlled temperature chambers for the specified exposure period. Seedings were spaced so that all treatments were completed at the same time. Then, all containers were moved to a warm room for completion of germination.

Germination data were recorded as percentages of healthy seedlings and transformed for statistical analysis using the angular transformation (9, 10). The data are presented as angular degrees and as percentages, with the least significant differences presented in degrees only. Analysis of variance was used to determine significant differences.

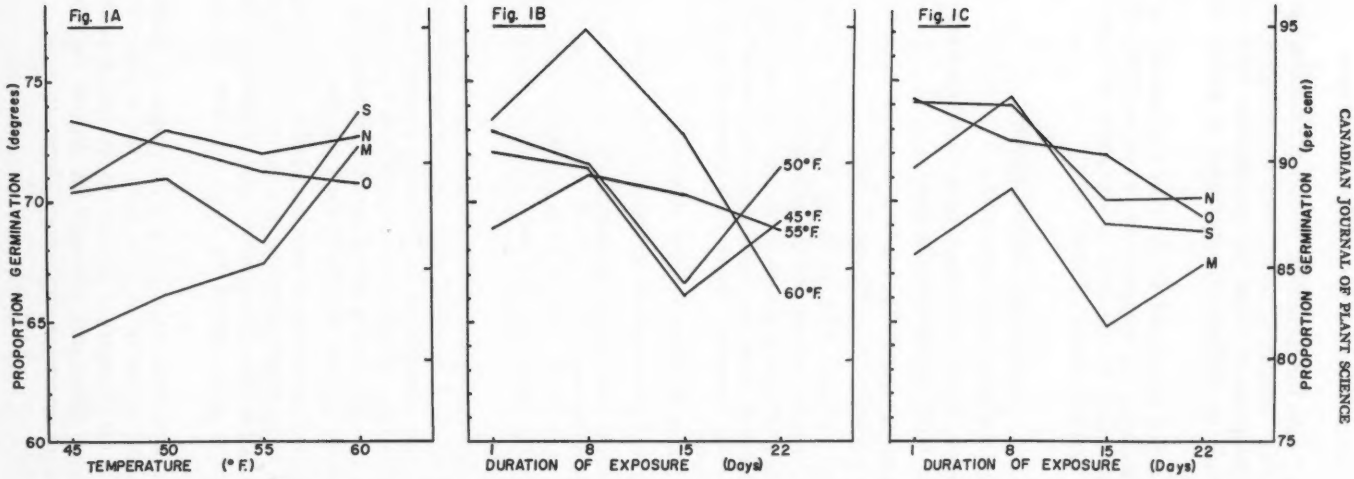

FIGURE 1. The effects of various interactions upon the germination of cucumbers: (A) variety x temperature; (B) temperature x duration of exposure; (C) variety x duration of exposure. (Variety code: N — National Pickling; O — Ohio MR17; M — Marketer; S — Straight 8).

EXPERIMENTAL RESULTS

On the completion of the exposure periods it was observed that a large proportion of the seed from all three test crops had germinated after exposure to 60°F. for 15 days or 22 days and to 55°F. for 22 days. Additional healthy seedlings were observed and included in the final count following 5 days' exposure to room temperature. Despite the use of a fungicide, there was considerable mould growth in both the cucumbers and watermelons germinated at 60°F. for 22 days.

Cucumbers

The cucumber germination data revealed significant differences due to varieties but none due to temperature or duration of exposure (Table 1). In Table 1A, it may be observed that only the germination of Marketer differed significantly from that of National Pickling.

The significance of two first order interactions indicated differences in the response of varieties to temperature treatment (Figure 1A) and in the effects of duration of exposure upon germination at different temperatures (Figure 1B). Figure 1A illustrates that the two pickling varieties, National Pickling and Ohio MR17, followed similar trends in germination except at the 45°F. temperature. Straight 8 behaved in the same manner up to 55°F., but germination increased sharply at 60°F. Marketer germination increased progressively as temperature increased.

Figure 1B shows that at 45° and 50°F. germination was highest after the 1-day exposure period, decreased progressively after 8 and 15 days, and increased again after 22 days. At 55° and 60°F. germination was highest after 8 days, decreased thereafter, and was lowest after 22 days.

As shown in Figure 1C, the variations in germination due to duration of exposure are similar for the four varieties.

Muskmelon

Analysis of the muskmelon germination data showed significant differences due to variety and temperature but none due to duration of exposure (Table 2). The varietal differences, shown in Table 2A, indicate that germination of Sugar Rock and Hales Best was significantly higher than that of Farnorth. Germination at 60°F. was significantly higher than at the other temperatures (Table 2B). Lowest germination was obtained after 15 days' exposure but this did not differ greatly from the germination at the other durations of exposure (Table 2C).

Varietal germination was not differentially affected by temperature (Figure 2A), as the interaction between these factors was not significant. However, the effect of temperature was significantly altered by duration of exposure, as shown in Figure 2B. At 45° and 50°F., germination decreased as duration of exposure lengthened to 15 days and increased sharply after 22 days. At 55° and 60°F., germination was highest after 8 days and decreased gradually to 22 days. As shown in Figure 2C, the duration of exposure differentially affected the germination of the four varieties. Only Hales Best and Cornell Delicious showed similar trends, but that of the latter was much more exaggerated.

TABLE 3. — MEAN GERMINATION OF WATERMELONS (IN DEGREES) AS AFFECTED BY (A) VARIETY, (B) TEMPERATURE, AND (C) DURATION OF EXPOSURE

A		B		C	
Variety	Mean germination	Temperature	Mean germination	Days exposed	Mean germination
		°F.			
New Hampshire Midget	53.6 (64.8)[3]	45	58.4 (72.6)	1	64.1 (80.9)
Sweet Siberian	52.8 (63.5)	50	59.8 (74.7)	8	63.7 (80.4)
Sweet Sensation	65.4[1] (82.7)	55	59.6 (74.4)	15	54.9[2] (67.0)
Early Canada	67.8[1] (85.7)	60	61.8 (77.7)	22	57.0[2] (70.4)
L.S.D. (0.05)	±1.7	L.S.D.	n.s.	L.S.D. (0.05)	±4.9

[1]Differs significantly from germination of New Hampshire Midget
[2]Differs significantly from germination after 1 day's exposure
[3]Per cent germination in parentheses

Watermelons

Analysis of the watermelon germination data revealed significant differences due to variety and duration of exposure but not due to temperature (Table 3). Table 3A shows that Sweet Sensation and Early Canada germinated significantly better than New Hampshire Midget. Temperature effects are listed in Table 3B. As shown in Table 3C, exposure periods of 15 and 22 days resulted in significantly lower germination than did the 1-day exposure period.

The effect of temperature treatment upon germination of the four varieties differed significantly, as illustrated in Figure 3A. The germination of Early Canada and Sweet Sensation increased as temperature increased while that of Sweet Siberian decreased. The response of New Hampshire Midget was intermediate, showing a decrease at 55°F. and an increase at 60°F. The effect of temperature treatment upon germination was significantly altered by duration of exposure, as shown in Figure 3B. Although the effects of exposure time were similar for the 45° and 50°F. treatments, germination at 55°F. deviated markedly for the 1-day exposure, and germination at 60°F. deviated for the 22-day period. All varieties did not respond similarly to duration of exposure, but differences were not significant (Figure 3C).

DISCUSSION AND CONCLUSIONS

The results of this study reveal certain similarities in the response of the three vine crops to the treatments imposed. Significant differences in varietal germination were observed within each crop. This was anticipated, since maturity of seed and inherent germinability can vary from one variety or seed lot to another.

Differences in germination due to temperature were significant only in the muskmelon trial, but certain trends were common to all three crops. In each case, the proportion of healthy seedlings was highest at 60°F., while differences in germination at the other three temperatures were small.

The effect of duration of exposure upon germination was significant only in the watermelon trial, in which the highest germination was obtained after the 1-day exposure and lowest after 15 days' exposure. Cucumbers and muskmelons also produced fewest seedlings after 15 days, but with these crops the differences were not significant.

All varieties of cucumbers and watermelons reacted similarly to the effects of duration of exposure, as all germination curves follow the same general trend (Figures 1C and 3C). Muskmelons were not consistent, as shown in Figure 2C. For example, Sugar Rock was little influenced by exposure time, while the germination of Cornell Delicious increased at 8 days, decreased at 15 days, and increased sharply after 22 days.

The similarity in germination curves (Figure 2A) showed that the germination of muskmelon varieties was not materially affected by temperature differences in the range from 60° to 45°F. The watermelons and cucumbers, however, reacted differentially. In one variety of each crop germination decreased as temperature increased, while in other varieties germination increased over the same temperature ranges. A third group reacted erratically (Figures 1A and 3A).

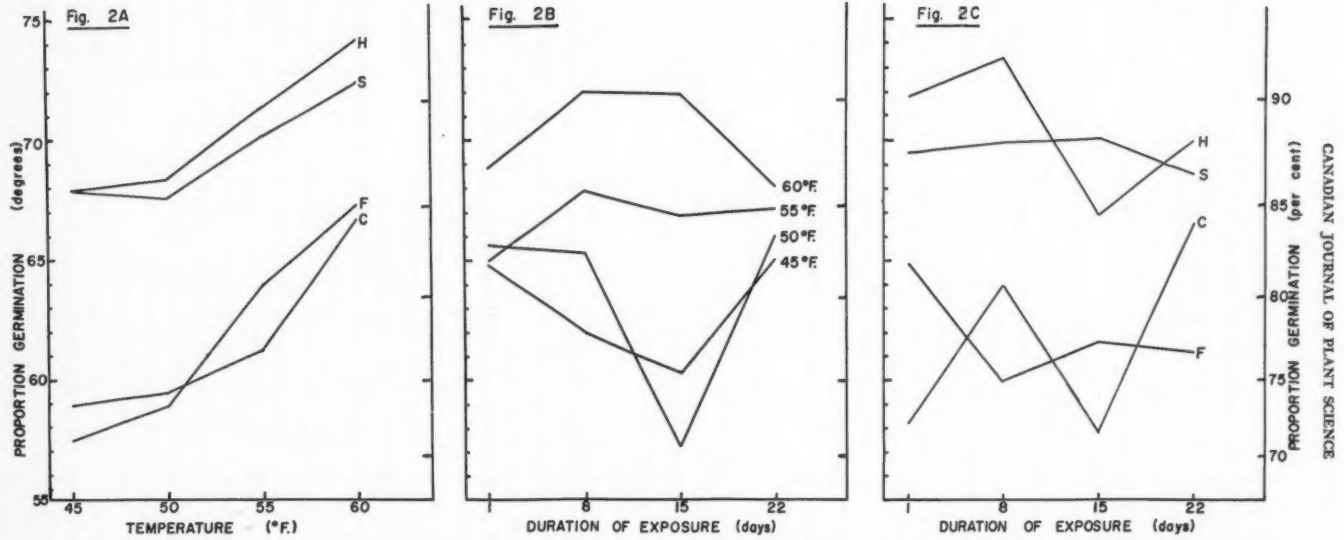

FIGURE 2. The effects of various interactions upon the germination of muskmelons: (A) variety x temperature; (B) temperature x duration of exposure; (C) variety x duration of exposure. (Variety code: H — Hales Best; S — Sugar Rock; F — Farnorth; C — Cornell Delicious).

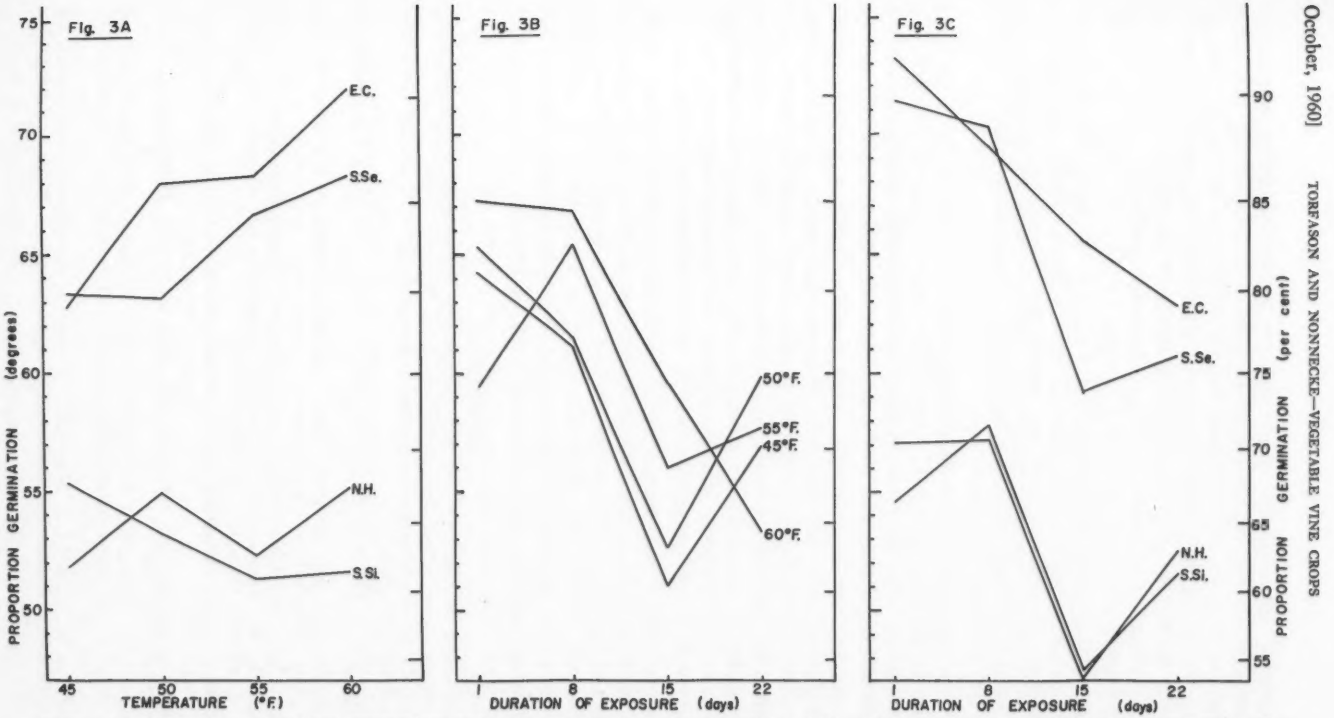

FIGURE 3. The effects of various interactions upon the germination of watermelons: (A) variety x temperature; (B) temperature x duration of exposure; (C) variety x duration of exposure. (Variety code: N.H. – New Hampshire Midget; E.C. – Early Canada; S. Se. – Sweet Sensation; S. Si. – Sweet Siberian).

It should be noted that the interaction between duration of exposure and temperature was significant in all three trials, indicating differential response in all cases. Here again, certain similarities in response are apparent. For example, germination at 45° and 50°F. decreased as exposure increased from 1 to 15 days and then increased sharply at 22 days. At 55°F., germination increased at 8 days, decreased gradually as the exposure period lengthened to 15 days, and levelled off after 22 days. At 60°F., the germination trend was similar to that at 55°F. up to the 15-day period, after which it decreased sharply at 22 days (Figure 1B, 2B, and 3B).

The decrease in germination at 60°F. and 22 days was not surprising, since the appearance of mould in the cucumbers and watermelons indicated that a decrease might result.

When the duration of exposure was increased from 15 to 22 days, the increases in germination at 45° and 50°F. were unexpected. Under these conditions, continued decreases in germination could be anticipated. However, all three crops reacted in the same manner, and it is improbable that this could be mere coincidence. It is postulated that a process of conditioning may have contributed to the recorded results. Such a process could have caused the formation of a stimulatory substance that required more than 15 days to attain a critical concentration. On the other hand, the formation of an inhibitory substance may have caused the pronounced decrease in germination at 15 days. Later, this substance may have become sufficiently depleted that there was little or no inhibition after 22 days.

In conclusion, it is apparent that cucumber, muskmelon, and watermelon seed will germinate satisfactorily after relatively long periods of exposure to temperatures much lower than the previously accepted minima. Germination, under field conditions, could be expected to be somewhat lower than that obtained in this study, but with optimal moisture and fungicidal control this difference should not be large.

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MEASUREMENT OF CHIPPING QUALITIES IN MANITOBA-GROWN POTATOES[1]

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ABSTRACT

Of ten varieties of potatoes from three major producing areas of Manitoba, Cherokee, Irish Cobbler, Norland and Netted Gem each produced high quality chips in at least one area. Variations in chipping qualities occurred between localities. Reducing sugar and specific gravity were reliable indexes of chip quality. A simplified method for measuring chip colour is outlined.

INTRODUCTION

The potato processing industry has rapidly gained in importance during the past decade. In the United States this industry now uses about one-fifth of all potatoes produced for food (10). The same trend has been evident in Canada.

The role of sugars in determining the quality of potato chips has been extensively studied since the initial work of Sweetman (9). The level of reducing sugars in potatoes has been reported to be the most important single factor influencing the colour of the chips (2-5, 11). The accumulation of these reducing sugars in potatoes during storage depends largely on variety and temperature (2, 8, 9). A Hunter colour and colour difference meter has been used recently (3, 4, 7) to measure potato chip colour objectively and thus provide an index of chip quality. High specific gravity has been reported to be associated with frying quality (6) and high chip yield (11).

This is a study of the measurement of chipping qualities of several varieties grown in major potato-producing areas of Manitoba. Special emphasis was given to the measurement of chip colour. Other quality factors included were reducing sugar content and specific gravity of the raw tubers and yield of the processed chips.

MATERIALS AND METHODS

Ten varieties of potatoes were grown at three localities in Manitoba during 1958. These included: Cherokee, Columbia Russet, Huron, Irish Cobbler, Keswick, Manota, Netted Gem, Norland, Pontiac and Waseca. The localities and soil types were: *Carman*, light sandy loam; *Fort Garry*, heavy clay; and *Poplar Point*, medium clay loam. The same source of seed was used for each variety at all the localities.

Chipping qualities of the potatoes were determined after 3 months of storage at 40°F. and again after 6 months.

The tubers were conditioned for 10 days at 65°F. prior to testing. Fifteen-pound lots of each variety from each locality were then immersed in succession in a series of seven brine solutions varying in specific gravity from 1.058 to 1.106. The floating tubers were removed from each brine

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tank and weighed. From these data the mean specific gravity of each lot was calculated. Only the potatoes in the median range of specific gravity were used for chemical analysis and chip preparation. This was desirable because of the heterogeneity of specific gravity found in each lot. Reducing sugar content was ascertained by a Lane-Eynon volumetric method (1).

The chips were prepared as follows: The tubers were washed, peeled and cut into 1/16-inch slices. Quadruplicate 100-gram portions of slices were prepared from each sample lot. Each 100-gram portion was rinsed in cold water to remove the surface starch and then placed between cotton towels to absorb the excess moisture. The raw slices were then immersed in a high grade of hydrogenated vegetable shortening previously heated to 375°F. and fried for 2½ minutes. The chips were placed in an oven at 80°F. for 2 minutes to drain off the excess oil; then they were weighed and the chip yield was calculated in terms of the original weight of the slices.

The chips obtained from each 100-gram portion of slices were prepared for colour determination by blending with a 100-ml. aliquot of ethanol in a Waring blendor for 30 seconds. The mixture was filtered through No. 1 Whatman paper and the moist residue transferred to a viewing cell 2½ inches in diameter and 1½ inches high, with an optical glass bottom. The residue was mixed thoroughly with a wooden spatula to remove bubbles

TABLE 1. — CHIPPING QUALITIES OF 10 VARIETIES OF POTATOES
(Means for three localities and two storage periods)

Variety	Specific gravity	Reducing sugar, %	Chip yield %	Hunter L value
Cherokee	1.089	.70	31.8	41.8
Columbia Russet	1.082	1.19	31.2	34.0
Huron	1.084	.92	29.2	24.7
Irish Cobbler	1.094	.56	33.9	47.4
Keswick	1.086	1.23	29.6	28.6
Manota	1.089	.66	32.2	40.2
Netted Gem	1.086	.60	33.1	44.6
Norland	1.084	.39	32.0	49.1
Pontiac	1.076	.94	28.0	33.3
Waseca	1.084	.69	30.5	34.0
Difference necessary for significance at 5% level	.005	.11	1.2	5.5

TABLE 2. — CHIPPING QUALITIES OF POTATOES FROM THREE GROWING AREAS IN MANITOBA
(Means for ten varieties and two storage periods)

Locality	Specific gravity	Reducing sugar, %	Chip yield %	Hunter L value
Carman	1.087	.69	31.3	41.3
Fort Garry	1.089	.71	31.9	41.8
Poplar Point	1.080	.96	30.2	33.3
Difference necessary for significance at 5% level	.003	.06	.7	3.0

TABLE 3. — CHIPPING QUALITIES OF POTATOES AFTER STORAGE FOR TWO PERIODS AT 40°F. (Means for ten varieties and three localities)

Storage period	Specific gravity	Reducing sugar, %	Chip yield %	Hunter L value
3 months	1.088	.73	31.2	39.2
6 months	1.082	.84	31.1	38.3
Difference necessary for significance at 5% level	.002	.05	—	—

and fine settlings from the viewing surface. Lightness of chip colour was determined on a Hunter colour and colour difference meter, the L circuit being used.

RESULTS AND DISCUSSION

The Hunter L values obtained on the chips were indicative of visual lightness of colour and were easily reproduced. A Hunter L value of over 50 was considered as good to excellent, 40 to 50 as fair, and under 40 as poor.

The varieties Cherokee, Irish Cobbler, Norland and Netted Gem each produced good-coloured chips (Hunter L value over 50) from at least one of the localities studied. Norland, Irish Cobbler and Netted Gem gave the best average chip colour (Table 1). Manota and Cherokee were intermediate in this regard. Norland had the lowest reducing sugar level and was the only variety averaging below the critical 0.4 per cent level reported by Hawkins *et al.* (5). Columbia Russet, Huron, Keswick, Pontiac and Waseca all produced chips with poor colour readings at each locality; reducing sugars were generally high for these varieties. Irish Cobbler had the highest average specific gravity and also the highest chip yield.

The chips produced from potatoes grown at Carman and Fort Garry were similar, on the average, in all qualities though the soil types were very different (Table 2). Poplar Point yielded tubers that were lower in specific gravity and higher in reducing sugar; and chips inferior in colour.

In storage, the specific gravity of the potatoes decreased significantly, the reducing sugar increased significantly, and the chip yield and chip colour remained practically constant (Table 3). The small but significant decrease in specific gravity during storage was attributed to loss of solids by respiration which apparently exceeded any dehydration effect. As the potatoes had been stored for 3 months at 40°F. before the tests, the rapid increase in reducing sugars generally occurring during initial storage at this temperature (8) was not apparent in these results.

The correlation coefficients for characteristics of the raw potatoes and chips for the two periods of storage at 40°F. were:

	After 3 months	After 6 months
Reducing sugar vs. Hunter L value	—.73**	—.87**
Specific gravity vs. chip yield	+.78**	+.78**
Specific gravity vs. reducing sugar	—.49**	—.52**

** Significant at 1% level

The correlation coefficients between reducing sugar and chip colour as determined by the Hunter L values agree favourably with that of Habib and Brown (3), who reported a coefficient of —.85 for the two factors. Specific gravity was a reliable index of chip yield; this supports the findings of Wright and Whiteman (11). The correlation between specific gravity and reducing sugar was negative and highly significant. In the present tests both the specific gravity of the tubers and their reducing sugar level were key factors influencing chip quality.

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ASSOCIATION BETWEEN SEED SIZE AND THE INCIDENCE OF LOOSE SMUT IN BARLEY[1]

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ABSTRACT

That small and medium sized seeds carry more loose smut (*Ustilago nuda*) (Jens.) Rostr. than large seeds in composite samples of barley was confirmed. The use of large seeds only for seeding purposes would be a practical means of reducing losses from loose smut. It is suggested that seed stocks to be used in breeding and testing procedures should each have the same proportions of small seeds removed to guard against misleading results.

INTRODUCTION

While average losses from loose smut (*Ustilago nuda*) in western Canada are not high, they do on occasion amount to 15 or 20 per cent in individual fields. Treatments are available that will control the disease, but these are generally impractical when large quantities of seed are involved. The restriction that any seed with over 4 per cent infection is ineligible for registration provides some measure of control. The main hope, however, in reducing losses from loose smut lies in the production of resistant varieties. Until resistant varieties are available in sufficient quantity to replace susceptible varieties at present in use, it would seem that any procedure that might result in reduced losses would be worthy of consideration.

In 1958 at Lacombe, Alberta, it was noted that plots sown with large kernels of Husky and Gateway barley produced fewer plants infected with loose smut than those seeded with small, medium or bulk seed. This was in agreement with results obtained by other workers. Taylor (3) in 1928 reported that small kernels of wheat carried three to five times as much loose smut as large kernels. Taylor and Harlan (4), working with four varieties of barley, showed that the smaller, lateral kernels of six-rowed barley carried an average of 13.4 per cent infection with loose smut compared with an average of 4.0 per cent infection in the larger, central kernels. They also showed that separation into different sizes resulted in the infected embryos being concentrated in the smaller seeded lots. With two varieties under test in 1938 and 1939 they obtained an average infection of 1.8, 5.4 and 12.8 per cent infection in large, medium and small seeds respectively.

Collaboration between the authors revealed that similar results had been obtained at Saskatoon in 1955 and plans were laid to further investigate the association of seed size with loose smut infection at Edmonton and Lacombe. This paper brings together results from these studies.

MATERIALS AND METHODS

Seed samples that showed natural infection with loose smut were secured for use in all experiments. Seed categories were established by

[1]Joint publication from the Experimental Farm, Lacombe, Alta.; Research Station, Saskatoon, Sask., and Plant Pathology Laboratory, Edmonton, Alta., Canada Department of Agriculture.
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sieve separation using appropriate slotted and round galvanized sieves for each lot of seed. The 1000-kernel weights of the seeds obtained appear in the tabulated results and indicate the relative size of seed in each sample. Smut infection was determined by the examination of embryos as described by Simmonds (2) or by the growth test. In one experiment both methods were used.

Two samples of Husky, two of Montcalm, and one each of Rex, Titan and Warrior were collected from farmers located in northeastern Saskatchewan for the study carried out at Saskatoon in 1955. Each sample was separated into large, medium and small sized seed lots and each lot was subjected to the embryo test for determination of infection. The two samples of Husky barley were also checked in a field growth test.

In the fall of 1958 a sample of Montcalm barley, reputed to be carrying 13 per cent infection, was secured for use in the studies at Lacombe and Edmonton. Using appropriate screens, this sample was divided into five groups according to size of seed and a sample of each, along with a sample of the bulk seed, was submitted to the Plant Products Laboratory at Saskatoon, Saskatchewan, for an embryo test for loose smut.

A growth test involving 200 plants from each of the six seed categories was conducted in the greenhouse at Lacombe. In this test, visual vigour ratings, ranging from one to five, with one indicating most vigour, were taken for all seedlings when they were in the one- to two-leaf stage to determine whether there was any association between seedling vigour, seed size and smut infection. A field growth test was also conducted in the summer of 1959 employing a randomized block design with 12-foot rows and four replications.

Further evidence of the effect of seed size on the incidence of loose smut was obtained from tests designed originally to study the effect of seed size on yield and yield components with Husky and Gateway barley.

TABLE 1. — CORRELATION BETWEEN SIZE OF KERNEL AND AMOUNT OF LOOSE SMUT INFECTION IN BARLEY VARIETIES AS DETERMINED BY THE EMBRYO TEST
Test conducted in Saskatoon, Sask., in 1955

Sample No.	Variety	Percentage smut and 1000-kernel weights					
		Large		Medium		Small	
		% Smut	Wt. in grams	% Smut	Wt. in grams	% Smut	Wt. in grams
1	Husky	4.0	34.3	4.9	25.7	10.3	22.2
2	Husky	1.0	38.6	4.1	33.7	6.4	24.8
3	Montcalm	3.8	45.0	8.8	35.0	15.8	27.0
4	Montcalm	2.0	48.7	17.5	31.5	28.8	17.7
5	Rex	6.7	46.0	11.4	31.0	13.7	25.0
6	Titan	3.1	51.0	9.3	40.0	14.5	29.0
7	Warrior	1.7	52.0	4.6	38.0	4.7	26.0
Average		3.2	45.1	8.7	33.6	13.5	24.5

The lots seeded with large, medium, small and bulk seed in these tests showed varying amounts of loose smut. Actual counts of smut-infected and healthy plants were made in the two centre rows of four rod-row plots with six replications. Both Husky and Gateway were infected in 1958 while only Husky showed infection in 1959.

RESULTS AND DISCUSSION

In all experiments conducted at Saskatoon (Table 1), Lacombe and Edmonton (Tables 2 and 3), small and medium sized seeds carried more loose smut than large seeds. These results agree with those of other workers and since seed stocks under study were from diverse regions and produced in different years; it would appear that this association between size of seed and smut infection is universal. The results at Saskatoon (Table 1) with Rex, the only two-rowed variety in the test, are of special interest. They suggest that the relationship of infection to seed size is applicable to two-rowed varieties as well.

On the whole, the relative infection in the various seed categories is fairly constant, even though the degree of infection varies from sample to sample. The average infection in large seeds was only 24 per cent of that in small and 37 per cent of that in medium, while that in medium sized seeds was 64 per cent of that in small.

Close agreement between embryo test and growth test results was obtained from the special tests conducted at Saskatoon. With Sample 1, the growth test showed 5.0, 9.5 and 11.8 per cent infection for large, medium and small seeds respectively compared to 4.0, 4.9 and 10.3 per cent for the embryo test reported in Table 1. With sample 2, the results were even closer. The growth test showed 1.9, 4.2 and 6.6 per cent infection and the embryo test showed 1.0, 4.1 and 6.4 per cent for large, medium and small seeds, respectively.

TABLE 2. — CORRELATION BETWEEN SIZE OF SEED AND THE INCIDENCE OF LOOSE SMUT IN MONTCALM BARLEY, AS SHOWN BY EMBRYO TESTS AND GROWTH TESTS

Class of seed	Percentage of total stock	1000-kernel wt. in grams	Percentage of smut infection		
			Embryo test (1)	Growth test (2)	Growth test (5)
Bulk	100.0	33.4	12.5	15.3	5.15
Large	16.0	46.0	2.7	2.1	0.87
Medium 1	38.3	38.1	9.7	12.8	4.08
Medium 2	31.0	31.4	18.8	12.6	6.16
Small 1	9.4	24.0	27.0	29.4	8.06
Small 2	5.3	23.2	23.0	30.5	7.25
Significant difference (5%)					1.78

(1) Embryo tests conducted through the courtesy of L. Bell, District Supervisor, Production Services, Plant Products, Canada Department of Agriculture, Saskatoon, Sask.
(2) Greenhouse test conducted at Lacombe, Alta., in fall of 1958
(3) Field test conducted at Edmonton, Alta., in summer of 1959

TABLE 3. — CORRELATION BETWEEN SIZE OF SEED AND THE INCIDENCE OF LOOSE SMUT IN HUSKY AND GATEWAY BARLEY GROWN IN THE FIELD AT LACOMBE, ALTA., IN 1958 AND 1959

Class of seed	1000-kernel wt. in grams	No. of smutted heads in 12 rod rows	% Smut infection
	1958		
	HUSKY		
Bulk	37.5	119	4.21
Large	48.0	18	0.64
Medium	36.9	105	3.72
Small	26.9	180	6.37
	GATEWAY		
Bulk	35.8	18	0.56
Large	43.3	7	0.22
Medium	34.7	19	0.59
Small	24.4	50	1.55
	1959		
	HUSKY		
Bulk	37.4	78	1.83
Large	48.6	8	0.18
Medium	36.7	84	2.00
Small	22.9	199	4.72

The embryo test and growth test results for the sample of Montcalm barley (Table 2) also show close agreement between infection as determined by the embryo or growth test. This is also in agreement with results previously reported by Russell (1). Results from the field test conducted at Edmonton show lower percentages in all seed classes, yet the relationships between the classes agree closely with the relationships from the embryo tests. General observations indicate that smutted plants produce fewer tillers than healthy plants and this could explain in part the lower percentages obtained, since head counts were made to determine infection. Further, mortality of infected plants could have been greater than in healthy plants, which would also contribute to a lowering of the percentage of infected heads.

The vigour ratings taken on the Lacombe test indicated that both size of seed and smut infection affected the vigour of seedlings. In general, healthy seedlings grown from small seeds showed less vigour than those grown from larger seeds. An average vigour rating of 2.93 was recorded for healthy seedlings grown from large seeds compared with 3.43 for healthy seedlings grown from small seeds. That infection affects seedling vigour was indicated by an average rating of 3.16 for healthy seedlings from all sizes compared with an average rating of 3.66 for infected seedlings from all sizes. Infected seedlings from large seeds showed a rating of 3.25 compared with 3.77 for infected seedlings from small seeds.

Table 3 presents results obtained with Husky and Gateway barley. Of special interest is the close agreement of results for both years with Husky, even though the source of seed was different. The Husky seed used

in 1958 was produced at Saskatoon while the seed for Gateway in 1958 and Husky in 1959 was produced at Lacombe.

PRACTICAL APPLICATION

At present most of the varieties of barley grown commercially in Canada are quite susceptible to one or more races of loose smut. The hot water, water soak and anaerobic seed treatments have proven effective in killing the smut organism, but they require special care and are useful only when relatively small quantities of seed are involved. The only practical means, therefore, of controlling loose smut on a large scale is to use seed that is known to be free of infection.

The results of these experiments and other screening tests indicate that most samples of barley are made up of 20 per cent large, 50 to 65 per cent medium and 15 to 30 per cent small seeds. In practice most of the small seeds are removed by present seed cleaning processes. The results of these experiments suggest that the removal of the medium seeds through screening would further reduce the incidence of loose smut. This would mean that it would be necessary to process 500 pounds of bulk grain to obtain 100 pounds of grain for seed. In a year of heavy infection, when pedigreed seed with low smut infection was scarce, this practice might prove to be a very logical procedure. It would not only ensure the possibility of better yield but, because of fewer infected heads in the growing crop, the possibility of re-infection might be reduced.

In some years, registered seed of any particular variety might be in short supply because of rejection due to stocks carrying a little over the limit of tolerance (4 per cent loose smut). The above results indicate that it would be quite feasible to re-clean such stocks. By so doing it would render them acceptable as registered seed.

Finally, these results have practical application in plant breeding and testing programs. If one is relying on natural infections to screen for loose smut resistance, it is essential that all seeds produced on a single plant be grown to assure that infected kernels are not missed. In progeny testing and variety testing, the presence of infected plants would be meaningless unless seed stocks for all lines or varieties were processed to the same level. In other words, bias would be introduced if, for example, seed used for one line represented 50 per cent of a bulk lot of seed and the seed for a second line or variety represented 75 per cent. Spurious yield differences could be recorded, resulting from different amounts of smut infection, even though there might be no inherent difference in the reaction of lines or varieties to loose smut. The results presented herein suggest that considerable care must be taken in preparing seed samples for comparative trials.

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RELATIONSHIPS BETWEEN Ca:K RATIO, pH, AND PREVALENCE OF POTATO SCAB[1]

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ABSTRACT

A preliminary study of ten soils selected from the commercial potato-growing area showed that scabbiness was generally associated with high pH levels and with wide Ca:K ratios.

In a greenhouse study, the effect of Ca:K ratio was studied independently of pH by using calcium hydroxide and calcium sulphate as sources of calcium. Scabbiness was directly related to soil pH but was not related to Ca:K ratio.

INTRODUCTION

It has been established that the incidence of potato scab, caused by *Streptomyces scabies* (Thaxt) Waksman and Henrici, is related to the pH of the soil. Increases in soil pH through the range of 4.8 to 7.0 have been shown to result in increased scabbiness (1, 3, 6, 9). It is the opinion of some workers however that the ratio of calcium to potassium in the soil may be an important factor in the development of this organism. Schroeder and Albrecht (7) observed that liberal applications of potassium as contrasted to calcium, and vice versa, resulted in increased scabbiness and that excess potassium caused more scabbiness than excess calcium. Gries *et al.* (2) suggest that the altered Ca:K ratio resulting from the application of lime may be as important a factor in increasing scabbiness as the change in soil pH.

In order to study the relationship between Ca:K ratio, pH, and potato scab, a preliminary investigation was conducted on a number of soils from the commercial potato-producing areas of New Brunswick. This was followed by an experiment in the greenhouse in which the influence of the Ca:K ratio was studied independently of pH.

MATERIALS AND METHODS

Preliminary Study

Samples of surface soil were selected from fields on ten farms in the commercial potato-growing areas where scabbiness was reported to be (a) *severe*, (b) *mild*, and (c) *absent*. The measurement of soil pH, and extraction of exchangeable cations were made by procedures as outlined by Peech *et al.* (4). Exchangeable calcium and potassium in the extracts were measured by means of a Beckman DU spectrophotometer with flame attachment.

Greenhouse Experiment

A Carleton loam (8) was treated with calcium hydroxide as required to supply 0, 2.0, 6.0, and 12.0 m.e. of calcium per 100 grams of soil. Potassium chloride treatments supplying 0, 0.2, 0.5, 1.0, and 3.0 m.e. of potassium per 100 grams of soil were superimposed on the calcium treatments in a factorial arrangement. A second series of treatments was in-

[1]Contribution No. 22 from Research Station, Research Branch, Canada Department of Agriculture, Fredericton, N.B.

cluded in which calcium was supplied as calcium sulphate. The calcium and potassium salts were mixed with the soil which was placed in glazed gallon pots. The soil was inoculated with *S. scabies* by adding to each pot 1 pound of soil from the Canada Department of Agriculture Research Station scab plots. The treatments were replicated three times. The soil was moistened and incubated for 30 days at which time samples were taken for the determination of exchangeable calcium, exchangeable potassium, and pH. A fertilizer treatment equivalent to 2000 pounds per acre of 6-12-12 was applied in a band 2 inches below the surface. One potato plant of the Green Mountain variety was grown in each pot. The crop was harvested at maturity. An index of degree of scabbiness was obtained by counting the number of scab lesions and dividing this value by the yield of tubers in kilograms.

RESULTS AND DISCUSSION

Preliminary Field Study

The results of the preliminary field study as presented in Table 1 show that scabbiness was directly related to Ca:K ratio and pH. Statistical analysis shows however that these two factors themselves are related ($r = +0.54$). It is impossible, therefore, to evaluate their individual effects on the basis of the results presented.

TABLE 1.—RELATIONSHIPS BETWEEN PREVALENCE OF POTATO SCAB pH, AND CA:K RATIO IN POTATO SOILS
(Data are mean values for ten samples)

Degree of scabbiness	pH range	Ca:K ratio
Severe	5.0–6.6	16.8
Mild	4.9–6.2	10.4
None	4.7–5.8	7.8

Greenhouse Study

(a) *Effects of Treatments on Soil Properties*

The effects of treatments on exchangeable calcium, exchangeable potassium, and pH are shown in Table 2. Increasing rates of calcium hydroxide resulted in a progressive increase in pH and exchangeable calcium, but had no significant effect on the Ca:K ratio. The fact that this ratio was not altered is due to the increase in exchangeable potassium with increased rates of applied calcium hydroxide. This increase in exchangeable potassium has been observed by Peech and Bradfield (5) and by York and Rogers (11).

Calcium sulphate lowered the pH slightly, increased exchangeable calcium, and widened the Ca:K ratio, but had no effect on exchangeable potassium. The fact that this salt had no effect on exchangeable potassium supports the observation of York *et al.* (10).

(b) *Relationship Between Soil Properties and Occurrence of Scab*

The correlation coefficients relating soil pH and scabbiness of potatoes were (+0.94) where calcium hydroxide was applied and (+0.62) where

TABLE 2. — RELATIONSHIPS BETWEEN CA:K RATIO, pH, AND PREVALENCE OF POTATO SCAB IN A GREENHOUSE PROJECT

Treatment m.e./100 gm. soil		Exchangeable Ca m.e./100 gm. soil	Exchangeable K m.e./100 gm. soil	Ca:K	pH	Scab lesions per kilogram of tubers
Ca	K					
Source of calcium — Calcium hydroxide						
0	0	5.5	0.19	28.9	5.2	128
0	0.2	6.0	0.22	27.3	5.2	93
0	0.5	6.1	0.24	25.4	5.2	131
0	1.0	5.6	0.27	20.7	5.0	90
0	3.0	5.4	0.70	7.7	5.0	53
Mean		5.7	0.32	22.0	5.0–5.2	99
2.0	0	6.7	0.21	31.9	5.5	74
2.0	0.2	6.6	0.22	30.0	5.5	154
2.0	0.5	6.9	0.28	24.6	5.5	247
2.0	1.0	6.3	0.37	17.0	5.4	241
2.0	3.0	6.3	0.82	7.7	5.3	137
Mean		6.6	0.38	22.2	5.3–5.5	171
6.0	0	9.3	0.22	42.3	5.9	464
6.0	0.2	9.8	0.26	37.7	6.1	416
6.0	0.5	9.5	0.72	13.2	5.9	474
6.0	1.0	8.7	0.96	9.1	5.9	564
6.0	3.0	7.9	2.05	3.5	5.8	528
Mean		9.0	0.84	21.2	5.8–6.1	489
12.0	0	13.8	0.56	24.6	6.8	611
12.0	0.2	13.1	0.55	23.8	6.7	933
12.0	0.5	11.3	0.65	17.4	6.6	765
12.0	1.0	10.6	1.03	10.3	6.5	630
12.0	3.0	12.5	2.09	5.6	6.5	708
Mean		12.3	0.98	16.3	6.5–6.8	729
Source of calcium — Calcium sulphate						
0	0	5.5	0.19	28.9	5.2	128
0	0.2	6.0	0.22	27.3	5.2	93
0	0.5	6.1	0.24	25.4	5.2	131
0	1.0	5.6	0.27	20.7	5.0	90
0	3.0	5.4	0.70	7.7	5.0	53
Mean		5.7	0.32	22.0	5.0–5.2	91
2.0	0	6.1	0.21	29.0	5.2	119
2.0	0.2	5.7	0.20	28.5	5.2	138
2.0	0.5	5.8	0.28	20.7	5.1	113
2.0	1.0	5.5	0.26	21.2	5.1	85
2.0	3.0	5.8	0.72	8.1	5.1	136
Mean		5.8	0.33	21.5	5.1–5.2	118
6.0	0	5.8	0.23	25.2	5.1	50
6.0	0.2	6.0	0.23	26.1	5.0	104
6.0	0.5	6.5	0.24	27.1	5.0	115
6.0	1.0	6.4	0.34	18.8	4.9	80
6.0	3.0	5.7	0.46	12.4	4.9	15
Mean		6.1	0.30	21.9	4.9–5.1	73

TABLE 2 (continued)

Treatment m.e./100 gm. soil		Exchangeable Ca m.e./100 gm. soil	Exchangeable K m.e./100 gm. soil	Ca:K	pH	Scab lesions per kilogram of tubers
Ca	K					
Source of calcium — Calcium sulphate (*cont.*)						
12.0	0	10.9	0.25	43.6	5.0	130
12.0	0.2	11.8	0.36	32.8	5.0	43
12.0	0.5	10.6	0.39	27.2	5.0	50
12.0	1.0	12.0	0.44	27.3	5.0	66
12.0	3.0	13.0	0.59	22.0	4.9	66
Mean		11.7	0.41	30.6	4.9–5.0	71

calcium sulphate was the source of calcium. The lower coefficient for calcium sulphate is due to the narrower range of pH values provided by this treatment for the calculation of the coefficient. In both cases however the relationship was significant at the 1 per cent level of probability.

The relationship between scabbiness and the Ca:K ratio was not statistically significant with either source of calcium.

It is concluded that the rise in soil pH resulting from the application of lime is the main factor influencing the occurrence and severity of scabbiness and that changes in the Ca:K ratio due to liming are not related to scabbiness.

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EFFECT OF LEAF RUST ON THE YIELD OF RESISTANT WHEATS[1]

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ABSTRACT

Heavy infection of leaf rust initiated at an early stage of plant development materially reduced the yield, kernel weight and bushel weight of one susceptible and three resistant varieties of wheat. The yield loss in the susceptible variety was 58 per cent whereas in the resistant varieties it ranged from 12 to 28 per cent. The results indicate a direct relation between the density of inoculum and the amount of necrosis on varieties that are resistant but not immune.

INTRODUCTION

Effective leaf rust resistance prevents yield loss by inhibiting reproduction and subsequent spread of the pathogen. In many instances, the necrotic areas associated with resistance involve the destruction of a certain amount of leaf tissue around each infection. Within a large area planted to resistant varieties, the spore population is kept at a low level and the total amount of dead tissue resulting from infection is also low. However, when resistant varieties are grown in experimental plots, the proximity of susceptible varieties provides a constant and heavy supply of inoculum and considerable destruction of leaf tissue of resistant varieties may occur. The authors have studied resistant varieties under these conditions in order to determine the yield losses resulting from such leaf destruction. It should be emphasized that this is an agronomically abnormal situation and the results do not give a true appraisal of the practical performance of the resistant varieties under field conditions.

METHODS

Four varieties were included in the test: Thatcher, highly susceptible to leaf rust (*Puccinia recondita* Rob. ex. Desm. f. sp. *tritici*), Selkirk, moderately resistant to leaf rust, and two highly resistant hybrids, Thatcher[7] x Frontana and Selkirk[8] x Exchange. Appreciable necrosis is associated with infections on Selkirk and the resistant hybrids. There were twelve plots of each variety, each consisting of four rod rows. Half of the plots of each variety were kept almost rust free by spraying them with Zineb (Dithane-Z78) (3) twice weekly and after every heavy rain from the time that rust first appeared in the plots until just before harvest. These plots received a total of eleven sprays. The remaining plots were not treated and became rusted by naturally occurring leaf rust inoculum. At maturity, the two centre rows were harvested and data were taken on yield, 1000-kernel weight, and bushel weight. The percentages of leaf rust and percentages of necrotic leaf areas were estimated while the leaves were still partially green. Diseases other than leaf rust were absent or insignificant.

RESULTS AND DISCUSSION

Leaf rust inoculum arrived early in the growing season and the sub-

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TABLE 1. — EFFECT OF LEAF RUST ON THE YIELD, KERNEL WEIGHT AND BUSHEL WEIGHT OF FOUR WHEAT VARIETIES

Variety	Treatment	Necrotic or chlorotic area of leaf (%)	Severity of infection — sporulating pustules (%)	Av. yield per acre (bu.)	Av. weight per 1000 kernels (gm.)	Bushel weight (lb.)
Thatcher	Sprayed	—	2 S	33.6	29.9	58.8
	Unsprayed	—	100 S	14.0	22.7	56.5
Thatcher[7] x Frontana	Sprayed	tr.	tr.	34.8	30.4	58.5
	Unsprayed	60	3 R	24.8	24.5	57.8
Selkirk	Sprayed	tr.	1 MR	33.6	37.8	56.8
	Unsprayed	40	20 MR	24.0	32.0	55.5
Selkirk[8] x Exchange	Sprayed	tr.	tr.	29.8	37.1	56.3
	Unsprayed	30	1 R	26.2	31.6	55.3
	Necessary difference, 5% level			5.9	1.14	1.0

sequent build-up on susceptible varieties was rapid. The varieties in this experiment were planted rather late and the combination of late planting and a rapid and early build-up of the pathogen resulted in very heavy infection of leaf rust at an early stage of plant development.

The results of the field experiments are summarized in Table 1. Zineb sprays gave excellent control of leaf rust on the susceptible variety and practically prevented necrosis on the resistant varieties. The rust readings shown in Table 1 were recorded at flowering time and leaf destruction progressed rapidly until the leaves were largely non-functional. The rate of leaf destruction was particularly rapid on Thatcher[7] x Frontana.

The resistant varieties suffered substantial yield losses. In fact, in two varieties, the yield loss was one-half that suffered by the susceptible variety. It is probable that the yield reductions in the resistant varieties were due to destruction of photosynthetic tissue. Destruction of photosynthetic tissue would also affect the yields of susceptible varieties. In addition, diversion of nutrients from the host to the fungus must be of considerable importance in affecting the yields of susceptible varieties. This appears to be particularly important during the period when seed set is being determined.

It is generally agreed that leaf rust causes yield losses by reducing the number of kernels per head as well as reducing kernel weight (1, 2, 4, 5). The stage of development reached by the plants before rust becomes severe determines whether reduced kernel production or reduced kernel weight is responsible for the major reduction in yield (5). In the present investigation, leaf rust was severe at a very early stage of plant development. Although the number of kernels produced per head was not estimated, the yield and kernel weight data indicate that reduced kernel production must have been an important factor in reducing the yield, particularly of the susceptible variety.

The present results show that yield tests with resistant varieties will be influenced by the proximity of susceptible plants. Under these conditions, the great advantage of the resistant variety, that of limiting the reproduction of the fungus, is largely nullified. However, these observations are not universally applicable since Caldwell *et al.* (1) found that a resistant variety used in their investigations did not suffer any yield loss. The extent to which the yield of a resistant variety might be affected under these conditions would in part be influenced by the type of resistance involved since the amount of necrosis resulting from each infection would determine the rate of leaf destruction. Certainly the present results show that extensive leaf necrosis caused by leaf rust diminished the yield, 1000-kernel weight, and bushel weight of the varieties which were studied.

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THE COMPETITIVE INTERACTION BETWEEN BARLEY PLANTS GROWN FROM LARGE AND SMALL SEEDS[1]

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ABSTRACT

The competitive effect on yield of plants grown from large and small seeds was demonstrated in both greenhouse and field tests. Plants from small seeds yielded approximately 77 per cent of those grown from large seeds in the greenhouse and 57 per cent in the field with inter-plant competition; with inter-row competition the percentages were 70 and 54 respectively; and, with no competition, 89 and 83 per cent respectively. It was further shown that increased competition favoured plants from large seeds. Superior production resulted mainly from a greater number of heads on plants grown from large seeds.

Suggestions are made as to how problems arising from this source of non-genetic variation could be overcome in various stages of selection.

INTRODUCTION

In 1956, considerable variation in root development was observed among seedlings of a pure line of barley although germination and environmental conditions were apparently uniform. Seed size was considered as a possible source of the variation and it was found that large seeds produced more vigorous seedlings than did small seeds (3). These results were in agreement with those reported from studies with sorghum (1) and grass species (4, 6). Further work at Lacombe indicated that large seeds produced plants with more tillers than those grown from small seeds (3, 5): an observation which was in accord with a previous report by Tedin as cited by Smith (7).

Large seeds also produced greater yields in an F_2 study in which plants from each seed size occupied a greenhouse block (5). It was assumed that the yield differences would have been greater had there been competition between plants grown from large and small seeds. To evaluate this hypothesis, experiments were conducted to study the effects of competition in the absence of genetic variation. Christian and Gray (2) reported that plants grown from large seeds of wheat had an advantage in inter-plant competition studies. The study on barley reported herein included both inter-plant and inter-row competition so that results could be applied to single plant and line selection. As propagation and testing are generally conducted in the field and greenhouse both environments were included in the study.

METHODS

Greenhouse and field tests were conducted to study 1) the effect of inter-plant competition between spaced plants from large and small seeds, 2) the effects of inter-row competition, and 3) the performance of plants in control tests where there was no competition between plants grown from the two seed categories.

A selection from Br 3902, a six-rowed hybrid, was used in all tests. Seeds were prepared by sieve separation; those that passed over a 7/64" x

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3/4" sieve were designated "large" and whole seeds that passed through a 1/12" x 1/2" sieve were designated "small". The approximate 1000-kernel weights of the two classes were 50 and 24 grams respectively.

A. *Greenhouse Tests*

The first series of greenhouse tests was conducted during the winter of 1959 and the second during the summer. Except where otherwise indicated, plant spacings were 4 inches within rows spaced 6 inches apart in planting beds.

To study the effect of inter-plant competition, large and small seeds were alternated within rows using 60 seeds of each category in two tests. The effect of closer competition was studied in a supplementary test with 2-inch spacings between plants. In this test, 120 seeds of each size were sown.

To study the effect of inter-row competition, rows of large and small seeds were alternated. Each seed class was represented by 48 plants in both series.

Large and small seeds sown in alternate beds to eliminate competition constituted the control test conducted during the winter. In the summer, plants were grown in 8-inch pots. Sixty plants from each seed size were grown in these tests.

Observations on plant development and disease reaction were made from emergence to maturity. All plants in tests where competition was a factor, and a few in the control tests, were rated at maturity on coronal root development. The number of seed-bearing heads was recorded for individual plants. Seed yields and 1000-kernel weights were taken after the plants from within seed classes were grouped and threshed.

The greenhouse experiments were arranged systematically rather than at random. For this reason, the results were not analysed statistically.

B. *Field Tests*

Field tests were conducted, in 1959, in an area carefully selected for uniformity. Before seeding the test involving inter-plant competition, the soil was worked with a rotary tiller, levelled and packed. Large and small seeds were alternated at 4-inch intervals in both directions in four 3′ x 12′ blocks. Care was taken to seed at a uniform depth of 2 inches. After seeding, irrigation water equivalent to one-half inch of rain was applied by sprinkler to assure sufficient moisture for germination. Plants around the perimeters of the blocks were discarded as were those surrounding the areas of missing plants. Head counts and general observations were made on 304 plants from each seed size. The plants in the respective categories were grouped before threshing and seed yields and 1000-kernel weights were obtained from the total lot in each group.

To study the effect of inter-row competition, plants from large and small seeds were grown in alternate rod-row plots spaced nine inches apart. Ten plots of each seed category were sown and equal numbers of seeds were used in each plot. Seedlings were counted at the one- to two-leaf stage on the area to be harvested. Observations were made from time of emer-

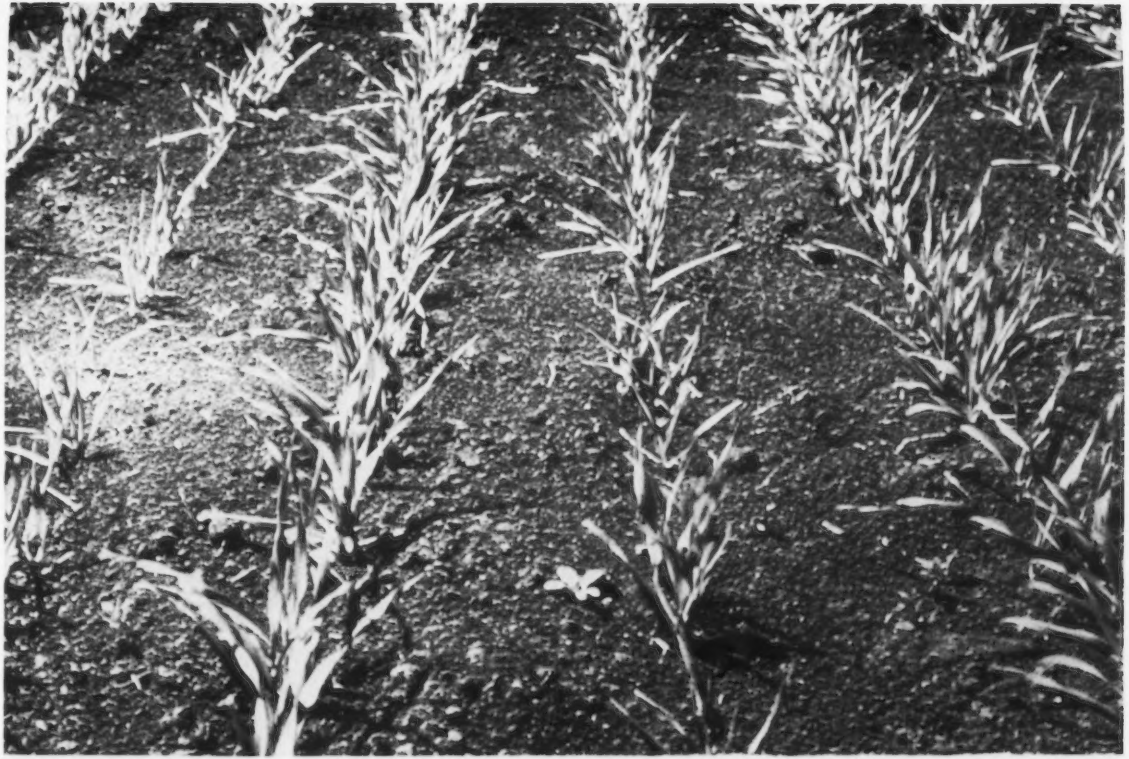

FIGURE 1. Greater vigour is exhibited by plants from large seeds in a rod-row field test. Photograph taken about 3 weeks after emergence.

gence to maturity on type of growth, plant development and disease reaction. Head counts and seed yields were recorded for individual plots and 1000-kernel weights were taken from the bulked seed.

The experimental materials in the two above tests were arranged systematically rather than at random. For this reason, the results were not analysed statistically.

The same general methods were employed in the control (no competition) test as in the test with inter-row competition, the chief exception being in plot type and arrangement. Four rod-row plots were used, for the control, in a completely randomized design with ten replications. Rows and plots were spaced 9 inches apart. Information was obtained from the two centre rows.

When the four-rowed plots sown to large and small seeds were contiguous, the guard rows were harvested to obtain information on seed yields and 1000-kernel weights as influenced by this "partial" competition. There were ten pairs of such adjacent rows.

The yield results of the last two of the above tests were analysed statistically.

The threshed seed of all field tests was screened to determine if the size of planted seed had an influence on the seed size of its progeny.

RESULTS AND DISCUSSION

A. *General Results*

In all tests, plants grown from large seeds were initially more vigorous than those grown from small, although time of emergence was virtually the same. Figure 1 illustrates the marked difference in vigour about 3 weeks after emergence in the field. The more rapid growth rate was manifested in earlier development of second and subsequent leaves and of tillers. Plants from large seeds headed 3 to 4 days and ripened about 2 days earlier than those from small. No differences between categories were noted in disease reaction.

B. *Greenhouse Results*

The results from corresponding winter and summer tests in the greenhouse were in close agreement. Because of seasonal yield differences this is best illustrated by comparing the ratios *large*:*small* presented in Table 1. The respective yield ratios for winter and summer tests with inter-plant competition were 1:.76 and 1:.78 and for inter-row competition 1:.70 and 1:.70. In the control tests the ratios were 1:.86 and 1:.93 respectively for the winter and summer tests.

The competitive advantage of plants from large seeds is also indicated in the above ratios since yield differences were greater between the two seed classes in tests involving competition than in the control tests. That the advantage increased as competition increased seems evident when the yield ratio 1:.54 for the test with 2-inch spacings in inter-plant competition is compared with 1:.78 for plants spaced 4 inches apart.

There was a close relationship between number of heads per plant and seed yields, particularly in summer tests involving competition. The yield

TABLE 1. — GREENHOUSE RESULTS SHOWING THE EFFECTS OF INTER-PLANT AND INTER-ROW COMPETITION BETWEEN PLANTS GROWN FROM LARGE AND SMALL SEEDS

Seed used	Seed bearing heads per plant	Seed yield per plant, grams	Root ratings[1]
		Inter-plant competition	
Winter test:			
Large	4.50	7.58	1.87
Small	3.66	5.74	2.65
Ratio L:S	1.81	1:.76	—
Summer test:			
4″ spacing			
Large	4.65	7.15	2.98
Small	3.60	5.57	3.36
Ratio L:S	1:.77	1:.78	—
2″ spacing			
Large	3.25	4.97	3.30
Small	1.85	2.70	4.00
Ratio L:S	1:.57	1:.54	—
		Inter-row competition	
Winter test:			
Large	3.28	5.78	1.93
Small	2.52	4.05	2.43
Ratio L:S	1:.77	1:.70	—
Summer test:			
Large	4.79	7.92	2.79
Small	3.40	5.55	3.31
Ratio L:S	1:.71	1:.70	—
		Control	
Winter test:			
Large	3.43	6.82	—
Small	3.40	5.87	—
Ratio L:S	1:.99	1:.86	—
Summer test:			
Large	4.38	6.43	—
Small	4.44	5.97	—
Ratio L:S	1:1.01	1:.93	—

[1] Lowest rating indicates best root growth

ratio *large*:*small* for the summer tests involving inter-plant competition was, for instance, 1:.78 and the ratio for heads per plant was 1:.77. This indicates that the entire yield difference can virtually be attributed to additional heads per plant. This association was not so close in the winter test with inter-row competition where it appeared that larger heads on late tillers accounted for some of the yield difference. In the control tests larger heads seem to account for most of the difference in yield between large and small seeds. Kernel weight accounted for 6 per cent of the difference in the winter test with inter-plant competition; otherwise the influence of this component was negligible.

Root growth was closely associated with top growth, as plants with superior root development produced more heads. Sprague and Farris (8) also reported a close association for these characters. In the control tests, there were no differences in numbers of seed bearing heads, or in root growth between plants from the two seed classes.

TABLE 2. — FIELD RESULTS SHOWING THE EFFECTS OF INTER-PLANT, INTER-ROW AND PARTIAL COMPETITION BETWEEN PLANTS GROWN FROM LARGE AND SMALL SEEDS

Seed used	Seedling count per plot	Seed bearing heads per plot	Seed bearing heads per plant	Yield[1]	1000 kernel weight-grams
			Inter-plant competition		
Large	—	—	3.73	7.99	41.2
Small	—	—	2.55	4.56	38.7
			Inter-row competition (single rod rows)		
Large	146	272	1.86	70.8	39.8
Small	132	156	1.18	38.2	38.0
			Partial competition (contiguous guard rows)		
Large	—	—	—	63.7	39.5
Small	—	—	—	41.2	40.3
			Control (2 centre rows of 4-rowed plots)		
Large	290	494	1.70	61.1	38.4
Small	308	412	1.34	50.7	39.8

[1]Yield is expressed in grams per plot for inter-plant competition, otherwise in bushels per acre

C. *Field Results*

The effect of competition on plants from the two seed classes when grown in rod-row tests under field conditions is clearly evident from the data in Table 2. Plots sown to large seeds consistently outyielded those sown to small seeds. The yield differences of 22.5 and 10.4 bushels per acre for the test with partial competition and the control test respectively were shown to be highly significant. Although the test involving inter-row competition was not analysed statistically due to the systematic layout, there can be little doubt that the yield difference of 32.6 bushels per acre is real. It is of interest to note that while there is little difference in total yield between the three tests, the yields of plots sown to large seeds increased and those sown to small seeds decreased as competition increased.

As in the greenhouse, superior production of plants from large seeds resulted mainly from additional heads per plant. This can be clearly shown by using the data from the test with inter-plant competition where the same number of plants represented each seed class. In this test large and small seeds produced 3.73 and 2.55 heads per plant and yielded 7.99 and 4.56 grams per plant respectively. The ratio *large:small* for the former is 1:.68 and for the latter 1:.57 which indicates that all but 11 per cent of the yield difference can be accounted for by additional heads per plant. There is also a close relationship between heads per plant and yield in the rod-row tests where plant numbers per plot were not the same for the two seed classes.

It appears that kernel weights of harvested seeds contributed somewhat to yield differences, particularly in inter-plant and inter-row competition; however, only in the latter test was a greater portion of "large" kernels obtained by screening. In inter-plant competition the smaller heads ob-

served on late tillers of plants grown from small seeds would account for some of the yield difference.

At first glance it may appear that plant stands, as indicated by seedling counts, influenced yield results. However, it is unlikely that they had a serious influence, as yields were more closely associated with heads per plant than with seedling counts. The data further show that the number of heads per plant was influenced more by competition between seed classes than by plant stands. For example, although seedling counts per rod row in large seeded plots were the same in inter-row competition and in the control test, a higher number of heads per plant was produced under competition. Conversely, plants from small seeds produced more tillers in the control test in spite of a heavier stand. It would normally be expected that the heavier stand would limit tillering.

APPLICATION

Results from this study may be applied to all stages of selection and testing of barley, and possibly all cereal crops, when vigour and yield are under consideration. Results of tests involving inter-plant competition have a bearing on seed preparation for testing in early generations, since there is a wide range of seed size from a single head or plant of six-rowed barley. To virtually eliminate this source of non-genetic variation in the F_2, seed should be separated into different size groups prior to seeding. Selections could then be made from within each size group. Conditions for single plant selection from within lines in the F_3 and subsequent generations could be improved by using uniform seed for any line.

The vast differences in the performance of plants from two sizes of seeds in the tests involving inter-row competition would indicate that, in progeny testing, the quality of seed used could determine whether a line would be selected or rejected from the standpoint of apparent productivity. Lines grown from a good quality of seed would tend to perform better in competition than those grown from abnormally small or shrunken seed.

Because of the effect of competition, it should be advisable to use short plots with multiple rows in the field, rather than a single row of greater length, in progeny testing for vigour and yield. This would help to reduce or eliminate the effects of competition arising from differences in seed size or any other source.

Results from the control tests suggest that comparable grades of seed should be used for all variety and adaptation tests.

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RELATIVE AND ABSOLUTE ELECTROLYTIC CONDUCTANCE TESTS FOR FROST HARDINESS OF APPLE VARIETIES[1]

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ABSTRACT

A laboratory method for testing frost hardiness of apple trees is described. Results demonstrated that diffused electrolytes from pre-hardened twigs provided a reliable means of testing their hardiness. There was a definite association between increased diffusion of electrolytes from hardened tissues and increased injury of twigs. This association was found useful in establishing both the relative degrees of hardiness of three named varieties of apples, Antonovka, McIntosh and Northern Spy, and the absolute values for the ultimate frost resistance of their tissues. Thus, although the total conductance of electrolytes of the three varieties was about the same before the artificial freezing treatments, ranging from 900 to 1000 micromhos, the average survival and the conductance of diffused electrolytes were as follows: hardy Antonovka 71 per cent and 296 micromhos; less hardy McIntosh 53 per cent and 445 micromhos; and least hardy Northern Spy 23 per cent and 611 micromhos. Results also established quantitative values for the ultimate frost hardiness of apple trees, namely: no appreciable injury when the conductance from hardened tissues of twigs was within 200 to 250 micromhos or less. Conductance of electrolytes above 350 to 450 micromhos generally signified total killing; whereas intermediary readings indicated partial injury to twigs.

The method used also permitted a study of the effects of several factors considered of vital importance in: (a) developing frost resistance of overwintering plants such as seasonal maturity, cold hardening and dehardening of tissues, and (b) causing excessive frost injuries such as increasing intensities and rates of freezing temperatures. The validity of the used laboratory methods is discussed.

INTRODUCTION

The physical properties of the protoplasm appear to be the major factors involved in frost hardiness of plant tissues. The most important property of the protoplasm in frost resistance is its ability to prevent coagulation or some other mechanical injury following freezing (4). Since injury to plants, whatever the cause, always results in loss of semi-permeability of the protoplasmic membrane, this fact may be utilized to test frost hardiness (3). The amount of electrolytes diffused out of the tissues may be measured and is found to be proportional to the injury. Previous studies (8, 9, 10) have shown the utility of such methods in establishing relative ratings of hardiness of both shoots and roots of several woody species. In this present study an attempt has been made to extend the usefulness of this technique to the point where an absolute value could be determined for the ultimate frost hardiness of some apple varieties. The method was also tested as a means of assessing the effect of certain environmental factors on the hardiness of apple seedlings. The value of such laboratory tests as an aid in eliminating tender seedlings in apple progenies prior to field planting is self-evident.

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MATERIALS AND METHODS

During the summer of 1957 the varieties Northern Spy, McIntosh and Antonovka, all budded the previous summer on *M. robusta 5* rootstock, were grown outdoors in 8-inch metal containers. The plants remained outdoors till November 15, 1957, after which they were moved to a thermostatically controlled hardening room. While outdoors, the plants were exposed to 15 days with light frosts ranging from 14 to 32°F. Previous to the first laboratory freezing tests on October 21 to 24, 1957, there occurred temperatures on 4 days ranging from 14 to 32°F. The remaining 11 days with temperatures ranging from 14 to 32°F. occurred before the second freezing tests on November 12 to 15, 1957. The results of laboratory freezing tests which followed, namely November 26, 1957, and January 14, 1958, were based on the material taken from the hardening room. The apple varieties were thus uniformly grown, matured and hardened under the same conditions so that any differences in their hardiness, as evaluated in this study, may be mainly attributed to genetic factors.

The hardening room was maintained at a constant temperature of 32 ± 3°F. and equipped with standard warm white fluorescent light of 32.8 watts per square foot. The artificial illumination was regulated to conform with natural daylight by means of time-clocks. Trees from field and from the hardening room were tested for hardiness in a freezing chamber with control instrumentation to maintain desired low temperatures of both shoots and roots. These temperatures were lowered from the pre-existing to the desired levels at a rate of about 15 to 20°F. per hour and kept at that specific level for 24 hours. It was realized that the rate of change in temperature was faster than usually encountered in nature. However, as will be shown, the results of this rate of freezing compared favourably with results of varietal testing of apples which followed a slow

TABLE 1.—CONDUCTANCE OF DIFFUSED ELECTROLYTES OF APPLE SHOOTS EXPRESSED IN MICROMHOS AND IN PER CENT OF TOTAL CONDUCTANCE FOLLOWING BOILING FOR SPECIFIED PERIODS OF SUMMER GROWTH, AUTUMN MATURITY AND WINTER HARDINESS

Dates	Exposure to min. temp. °F.	Northern Spy		McIntosh		Antonovka	
		Micro-mhos	Per cent	Micro-mhos	Per cent	Micro-mhos	Per cent
Aug. 28, 1957	40	324	—	335	—	125	—
Oct. 10, 1957	29	300	31	190	23	145	16
Oct. 16, 1957	26	120	15	100	14	98	13
Nov. 6, 1957	20	132	—	118	—	113	—
Nov. 12, 1957	14	160	—	123	—	113	—
Jan. 13, 1958	14	176	14	187	15	143	12
Feb. 11, 1958	14	153	13	145	13	121	10
Apr. 21, 1958	14	210	—	170	—	130	—
July 9, 1958	—	1350	84	1245	87	1175	79
July 14, 1958	—	1080	68	950	60	875	71
July 21, 1958	—	1000	50	900	42	687	38
Aug. 5, 1958	—	1050	56	725	40	570	37

freeze at a rate of 1°F. per hour. For various reasons it was necessary to use the more drastic, rapid method of freezing the material.

The freezing unit was supplemented with an insulated box equipped with thermostatically controlled lead heating cable and styrofoam insulation. This enabled simulating outdoor winter minimum ground temperatures of 28 ± 2°F. which commonly occur in the root areas of surface soil under snow, while shoots independently were being subjected to specific freezing temperatures. The varietal differences in freezing injuries of such hardened plants were determined by their ability to grow when returned outdoors on April 16, 1958. The portion of the twig lacking bud growth, and having unhealthy discoloration and shrivelling of the bark and lack of flexibility on manipulation, was considered to have been killed. Such portions were measured in inches and were expressed as per cent of total length of twigs. These were photographed on July 30, 1958. The injuries were then related to the conductance of diffused electrolytes which had been determined immediately after each freezing treatment. In this manner, as will be shown, it was possible to establish both the relative and absolute values for conductance as a prediction of seedling hardiness.

To determine the conductivity of solutions of diffused electrolytes unwashed twigs were prepared in a manner previously used for testing both shoots and roots (8, 10). These were cut into half-inch segments to which was added an amount of distilled water, exactly five times the weight of the material, to allow the diffusion of electrolytes mainly from cut surfaces. This was left standing for 24 hours at room temperature after which the conductance of the exosmosed electrolytes in the water extract was measured at a temperature of 77°F. with a wheatstone bridge. Total electrical conductance of twigs was determined by boiling the segments in water for 2 minutes, restoring the water lost during boiling, left standing for 24 hours at room temperature and the conductance again measured at a temperature of 77°F. with a wheatstone bridge. The size of the box within the freezing unit permitted the freezing of only two plants of each variety during each treatment. The size of the sample was generally doubled when treatments did not require artificial freezing as in the case of check plants and certain outdoor treated plants tested during summer and autumn.

EXPERIMENTAL RESULTS

Effects of Seasonal Maturity on Hardiness of Fruit Trees

The effect of seasonal maturity and cold hardening on diffusion of electrolytes from tissues of hardy Antonovka, less hardy McIntosh and relatively tender Northern Spy (Table 1) showed that there was very little difference in the type of growth (Figure 1) or in the electrolytic conductance (e.c.) of the varieties during the active growing season of July 1958. At this time, the e.c. of the varieties ranged from about 800 to 1350 micromhos. Conductance of diffused electrolytes declined rapidly with advancement in maturity and duration of the cold hardening season. Thus, during the period of August 28 to November 12, 1957, before the plants were stored indoors, the decline in e.c. of the Antonovka was of a minor nature from 125 to 113 micromhos, indicating that the level had

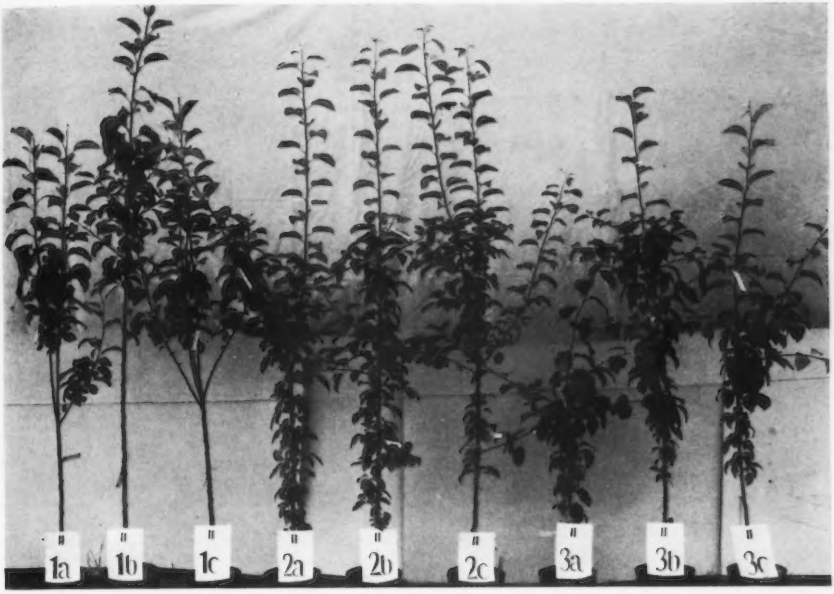

FIGURE 1. Development of apple varieties: 1. Northern Spy, 2. McIntosh, 3. Antonovka, during the growing season of 1958. These trees underwent a normal change in conductance of electrolytes which diffused from shoots during the laboratory tests ranging from about 1250 micromhos during active growth to about 125 micromhos when shoots were dormant and hardened (See Table 1).

FIGURE 2. Differences in shoot growth of: 1. Northern Spy, 2. McIntosh, 3. Antonovka, following artificial freezing at 0°F. on a. October 22, 1957, and b. January 14, 1958. The additional cold hardening decreased the injuries of twigs and the conductance of electrolytes which diffused from the tissues during the laboratory tests. (See Table 2).

FIGURE 3. Differences in shoot growth of: 1. Northern Spy, 2. McIntosh, 3. Antonovka, following artificial freezing on November 13, 1957, at a. 0°F., and b. −10°F. The additional cold temperatures had increased the conductance of electrolytes from tissues during the laboratory tests in proportion to the injuries of plants in field. (See Table 2).

FIGURE 4. Difference in shoot growth of: 1. Northern Spy, 2. McIntosh, 3. Antonovka following changes in temperatures from 32°F. to −30°F. at a. slow rate of about 1°F. per hour, and b. faster rate of 15 to 20°F. per hour. The additional rates of freezing had increased the injuries of shoots and the conductance of electrolytes which diffused from tissues during the laboratory tests. (See Table 3).

already been stabilized. In the McIntosh variety, there was a marked continued decrease throughout the period of August 28 to October 16, while in the Northern Spy the greatest decline occurred in the period of October 10 to 16. After October 16 and during the period of indoor storage from January 13 to April 21, 1958, relatively little change occurred in conductance of diffused electrolytes from tissues. The findings thus demonstrated the usefulness of the electrolytic method for distinguishing certain natural physiological changes considered of importance in increasing frost hardiness. These data also show that earliness of maturity may be associated with frost hardiness.

Varietal Differences in Hardiness of Apple Trees

A definite association was found between increased conductance of diffused electrolytes from hardened tissues and increased frost injury of twigs. This was useful in establishing the relative degree of hardiness of the three varieties of apples (Table 2). Thus, although the total e.c. of the three varieties was about the same before freezing treatments, ranging from 900 to 1000 micromhos, the average conductance (omitting check treatments) of electrolytes diffused from the hardier Antonovka with 71 per cent survival was 296 micromhos. This was less than the conductance of electrolytes from the relatively less hardy McIntosh with 53 per cent

TABLE 2.—CONDUCTANCE OF DIFFUSED ELECTROLYTES OF APPLE SHOOTS EXPRESSED IN MICROMHOS IN ASSOCIATION WITH PER CENT GROWTH SURVIVAL FOLLOWING TREATMENTS IN 1957-1958

Variety	Date treatment begun	Temperature treatments							
		Check		0°F.		—10°F.		—20°F.	
		Conductance in micromhos	Survival per cent	Conductance in micromhos	Survival per cent	Conductance in micromhos	Survival per cent	Conductance in micromhos	Survival per cent
Northern Spy	Oct. 22, 1957	120	100	789	0	733	0	874	0
	Nov.13, 1957	132	100	246	100	476	0	791	0
	Nov.26, 1957	160	100	323	85	666	0	871	0
	Jan. 14, 1958	176	100	236	100	484	56	841	0
	Mean	147	100	398	71	590	14	844	0
McIntosh	Oct. 22, 1957	100	100	540	0	584	0	849	0
	Nov.13, 1957	118	100	191	100	326	87	461	0
	Nov.26, 1957	123	100	250	95	436	85	686	0
	Jan. 14, 1958	187	100	183	100	346	99	486	70
	Mean	132	100	291	74	423	68	620	17
Antonovka	Oct. 22, 1957	98	100	190	70	340	0	716	0
	Nov.13, 1957	113	100	123	100	193	100	500	0
	Nov.26, 1957	113	100	141	100	190	100	408	91
	Jan. 14, 1958	143	100	166	100	226	100	356	90
	Mean	117	100	155	92	237	75	495	45

survival and 445 micromhos, and the least hardy Northern Spy with 28 per cent survival and 611 micromhos.

Generally no appreciable frost injury resulted to apple twigs when the e.c. from hardened tissues was within the range of 200 to 250 micromhos, or less. Conductance of electrolytes above 350 to 450 micromhos generally signified total killing of twigs whereas intermediary readings indicated partial injury to twigs (Figures 2, 3, 4).

The methods used revealed the importance of cold hardening in frost resistance of plants. In all varieties (Table 2), full maturity but not complete hardening of shoot tissues may have occurred by October 22 when the e.c. reading had been almost stabilized. This is illustrated by the fact that, when exposed to 0°F. at this date, there was no survival in Northern Spy and McIntosh and only 70 per cent survival in Antonovka (Figure 2). Yet, when exposed to the same temperature on November 13, November 26, and January 14 after a further period of hardening, the survival was about 100 per cent in all cases.

Obtained data showed that increasing the intensity of cold on November 13 from 0°F. to −10°F. resulted in a general increase of conductance of Northern Spy, McIntosh and Antonovka of 230, 135, and 70 micromhos respectively and increased injuries of 100, 13 and 0 per cent, respectively (Figure 3). These results give further evidence of the reliability of the methods used in this study to evaluate frost hardiness of plants.

Additional data on the influence of certain environmental factors, such as rates of temperature drop on frost injury of plants (Table 3), show that during April the relatively faster rates of freezing from 32°F. to −10°F. and −30°F. have resulted in a general increase of conductance of diffused electrolytes. These data (Figure 4) presented further evidence that generally no injury resulted to apple twigs when e.c. of hardened tissues was about 250 micromhos or lower. Conductance of approximately 500 micromhos or more signified total killing of twigs, whereas intermediary

TABLE 3.—CONDUCTANCE OF DIFFUSED ELECTROLYTES OF APPLE SHOOTS, EXPRESSED IN MICROMHOS IN ASSOCIATION WITH PER CENT GROWTH SURVIVAL FOLLOWING SPECIFIED FREEZING TREATMENTS IN 1958

Date	Temperature treatments		Variety					
			Northern Spy		McIntosh		Antonovka	
	Intensity	Rate of changes	Conductance in micromhos	Survival per cent	Conductance in micromhos	Survival per cent	Conductance in micromhos	Survival per cent
Apr. 14	−10°F.	Slow[1]	340	65	213	100	110	100
Apr. 17	−10°F.	Quick[2]	932	0	500	0	523	0
Apr. 21	−30°F.	Slow[1]	1030	0	489	50	246	100
Apr. 25	−30°F.	Quick[2]	1189	0	939	0	802	0

[1]Slow freezing maintained at an average rate of 1°F. per hour
[2]Quick freezing maintained at a rate of about 15 to 20°F. per hour

e.c. indicated partial recovery of 65 to 50 per cent. Conductance of electrolytes from shoots of Northern Spy, McIntosh and Antonovka, following slow freezing as compared with quick freezing, as an average of −10°F. and −30°F. temperatures, were 685, 351 and 178 micromhos and 1060, 719 and 663 micromhos, respectively. This indicated that, in general, both types of freezing treatments may be used to establish ratings of frost hardiness of hardened plants. Nevertheless, since slow freezing gave better differentiation between varieties in conductivity readings than quick freezing, it is possible that the slow freeze might be a more effective method for establishing small differences in hardiness of tissues and when tissues are not fully hardened as shown below.

A certain amount of dehardening had occurred in apple tissues during the period of storage of January 14 and April 17. (Compare Tables 2 and 3). The three apple varieties were subjected on both dates to a quick freeze of −10°F. Following the treatment in January, the e.c. and survival of Northern Spy, McIntosh and Antonovka were 484, 346 and 226 micromhos and 56, 99 and 100 per cent, respectively. Following the same freezing treatment in April, the e.c. and survival of the three varieties were 932, 500 and 523 micromhos with no survivals. This indicated that the quick freezes in April were too severe in April but not in January to establish tests of relative hardiness whereas such tests could be made in April more reliably with slow freeze treatments. It is apparent that a considerable amount of experimentation is necessary to pre-select suitable temperature treatments for an intended period of testing to differentiate hardy from tender varieties of plants.

DISCUSSION AND CONCLUSIONS

The findings of this study have demonstrated that conductance of diffused electrolytes from tissues provides a reliable means of determining relative and absolute ratings of hardiness of apple trees as well as a means of studying the effects of certain seasonal changes on their (plant) winter survival. The results have indicated that generally no injury resulted to apple shoots when the electrical conductance of diffused electrolytes from hardened tissues was below about 200 to 250 micromhos. Conductance of electrolytes above 350 to 450 micromhos generally indicated total killing. Intermediary conductance signified partial injury of shoots.

The outward diffusion of electrolytes from hardened tissues indicates mainly the condition of the semi-permeable membrane (3) because permeability is a property of the membrane and not of the substance which diffuses through it. Since injury to plants, whatever the cause, always results in loss of permeability, diffusion in excess of normal would indicate a critical internal condition of tissues and subsequent degree of injury to twigs. The usefulness of conductance readings of diffused electrolytes from hardened tissues as predictions of frost injury to overwintering plants is obvious.

The electrical conductivity method appeared also to be a good measurement of changes in permeability of living cells due to seasonal periodicity in vegetative growth. The findings of this study (Table 1) showed that

during a normal growing season there was a general decline in electrical conductance of new growth of shoots with seasonal changes from summer to autumn and late fall maturity. This may be due to the fact that young active protoplasm increases in viscosity as it matures and becomes less active (5). It may seem contradictory that an increase in permeability should accompany both increases in activity during the growing season and increases in frost injury. These increases, however, are not due to the same causes. Those due to frost injury, because of excessive cold (Table 2), and/or excessive rates of freezing (Table 3), may be due to the resultant destruction of the protoplasm (3, 7). Such injury may also be caused by desiccation during winter storage (9). A number of environmental factors on the other hand may increase the permeability of the membrane during the growing season, the most important of which may be temperature conditions. Results of this study support the findings of others (1, 2, 6) that on basis of conductance of diffused electrolytes from unfrozen fully mature and fully hardened living tissues, a lower permeability normally characterizes more winter hardy plants. By this method was established an association between earliness of maturity and frost hardiness of apple twigs (Table 1). Pre-hardening mature tissues by temperatures at or near freezing was found to be a means of increasing frost hardiness of twigs (Table 2). A certain amount of dehardening of apple tissues had occurred during the period of storage which decreased the hardiness of twigs (Tables 2, 3). These results thus suggest that the state of protoplasm which controls conductance of diffused electrolytes and determines the degree of frost hardiness of tissues is conditioned partly by environmental factors and partly by internal properties of plants.

The electrical conductivity test has been described by Levitt (3) as "an ingenious method of speeding up the measurement of frost hardiness and of making the numerical rating objective". The results of this study support this concept. These findings provide a laboratory method for eliminating tender seedlings in apple progenies and other closely related plants, prior to their field planting. These investigations also suggest the desirability of conducting intensive fundamental research on the differences in function and structure of the protoplasmic membrane which controls diffusion of electrolytes, as a major criterion of hardiness in over-wintering plants.

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WINTER HARDINESS EVALUATION IN ALFALFA

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ABSTRACT

A study was undertaken to determine the relationship between carbohydrate content in the roots of alfalfa varieties in the fall and winter hardiness of the varieties. Several agronomic characters were also considered in relation to winter hardiness. Winter injury was found to be inversely proportional to the hexosan content. Growth recovery in the fall was also associated with winter injury; the faster the recovery, the greater the winter injury of the variety. Rapid growth recovery and hexosan content were closely associated. Root development in the seedling year was slower in hardy varieties than in less hardy ones. It was concluded that a combination of the recovery characteristic and hexosan content in the roots could be used in screening alfalfa material for hardiness qualities. It is suggested that the roots of selections made from F_2 populations, originating from hybrids between hardy and non-hardy stocks, could be analysed for hexosan content in the fall and accordingly classified into hardiness groups. The roots could be used for this purpose, while stem cuttings could serve the propagation requirements.

INTRODUCTION

In the Prairie Provinces of Canada it is essential that alfalfa varieties be extremely hardy because the long cold winters, frequently variable as to snow cover and alternate thawing and freezing conditions, often cause severe winter-killing. A rapid evaluation for winter hardiness would be very helpful in a breeding program since nature often does not provide the right conditions for an evaluation just when the plant breeder wants it and, consequently, there is a delay in progress.

The numerous biochemical entities and physiological processes which have a bearing on the hardiness of plants have been adequately reviewed by Levitt (12). A number of methods for screening alfalfa into different hardiness classes have been suggested by several workers (7, 8, and 13) but so far none has been used extensively by plant breeders, mainly because the differentiation is inadequate within narrow ranges.

Dexter (7) followed the trend of cold resistance in three alfalfa varieties by electrical conductance and observed varietal differences during October and November. More recently Greenham and Daday (8) concluded that the electrical resistance index could be used for the rapid discrimination of cold-susceptible and cold-hardy strains. However, they were unable to show any significant differences between the variety Ladak and a Canadian creeping-rooted strain, although the latter is known to be more winter hardy than Ladak in Western Canada (9, 10). Bula, Smith and Hodgson (3) similarly showed that the electrical conductance test gave good discrimination between alfalfa varieties differing widely in hardiness qualities.

Rodger *et al.* (13) reported that differential germination in sugar or salt solution of specific concentration offered a possibility for classifying alfalfa strains into hardy and non-hardy types. However, Heinrichs (11) in using the method on a large number of varieties and strains of diverse genetic

origin concluded that, although there is a tendency for non-hardy varieties to germinate better than hardy ones in sugar and salt solutions, there are frequent exceptions to this tendency. Thus, the method would appear to have little value for screening breeding material in which hardiness differences vary within relatively narrow limits.

Over-wintering of alfalfa is known to be associated with carbohydrate storage and with changes in certain carbohydrate components. Bula and Smith (2) found that total available carbohydrates in roots of Ranger alfalfa reached a high around mid-October in Wisconsin. From mid-October to mid-December total sugars continued to increase, while starch decreased until a level of 6 per cent was reached in mid-February. In a later paper Bula *et al.* (3) reported that the total available carbohydrates were maintained at a much higher level in the fall by non-hardy Arizona Common than by a hardy *Medicago falcata* strain. Ranger had a carbohydrate content intermediate between these two. The trend was found to be the same in Alaska as in Wisconsin.

The present study was undertaken to determine the relationship between root carbohydrate content of alfalfa varieties in the fall and winter hardiness of the varieties. In addition, several agronomic characters were considered in relation to winter hardiness.

MATERIALS AND METHODS

Thirteen alfalfa varieties of diverse genetic origin and known to differ in winter hardiness were seeded in a 6-replicate test of the random block design in May, 1955, on loam soil at Swift Current, Saskatchewan. The rate of seeding was 6 pounds per acre in drill rows 12 inches apart. Plots consisted of six rows, 1 foot apart and 35 feet long. An excellent stand was obtained of all varieties and they made better growth during the establishment year than is normal for the region.

On October 1, 1955, the test area was mowed and all top growth removed. This was done at this late date because experience had established that taking the top growth off after this date does not contribute to winter injury of the alfalfa. On October 18 the varieties were rated for recovery on the basis of 1 to 5, where 1 represents rapid recovery and 5 very slow

TABLE 1.—THE RELATIONSHIP BETWEEN ABSORBANCES (Y) AND GLUCOSE FRACTIONS (X) IN S-1-N DERIVATIVES OF KNOWN GLUCOSE-XYLOSE MIXTURES.

(Total concentration of monosaccharides 40 mgm. per litre)

Wavelength	Regression equations $Y = a + bX$)
530	$Y = 0.771 - 0.379X^{**}$
540	$Y = 0.875 - 0.408X^{**}$
550	$Y = 0.898 - 0.354X^{**}$
560	$Y = 0.710 - 0.096X^{**}$
565	$Y = 0.566 + 0.080X^{**}$
570	$Y = 0.436 + 0.210X^{**}$
580	$Y = 0.260 + 0.291X^{**}$

*Significant at P = .05
**Significant at P = .01

recovery. The same day root samples were obtained for carbohydrate analyses and root weight determinations. Two samples, each 1 foot square by 6 inches deep, were taken on each plot. These samples were dug from outside rows so as not to interfere with observations made on the plots in future years. Root samples were again taken in mid-October, 1958, and analysed for available carbohydrate content.

The available carbohydrates were determined on the basis of hexosans and pentosans. The reaction of monosaccharides with purified sulphonated 1-naphthol (S-1-N) as reported by Devor (4) was modified by the second author to overcome the limitation of this method in the analysis of extracts with less than 15 per cent pentose in the soluble carbohydrates. These modifications to the procedure are discussed under the next section.

Estimates of winter injury were made each spring about mid-May at which time the damage could be adequately evaluated. The winter damage was based on stands of plants which were alive the previous fall.

Precipitation during the period of the test was above normal in 1955 and much below normal in the other 3 years. During the months April to September, inclusive, the precipitation in inches was as follows: 1955, 12.03; 1956, 7.85; 1957, 7.78; 1958, 7.48. The normal for this period is 10.22.

RESULTS AND DISCUSSION

According to Devor (5) one common absorption band exists in the visible spectrum for S-1-N derivatives of monosaccharides. All hexoses studied had a maximum absorption near 750 millimicron (mu) and all pentoses near 550 mu.

Since no evidence has been reported of interfering substances in extracts of various plant materials (4, 6), it was assumed that the S-1-N derivatives in alfalfa root extracts were predominantly hexoses and pentoses. This being the case, it was concluded that a "two component system" (14) was being dealt with.

TABLE 2.—WINTER INJURY OF ALFALFA VARIETIES IN FOUR SUCCESSIVE YEARS, ESTIMATED IN MAY OF EACH YEAR ON THE BASIS OF THE PREVIOUS YEAR'S STAND

Variety	Winter injury % mean 6 replicates				Winter injury index*
	1955-56	1956-57	1957-58	1958-59	
Siberian-Sc. 1522	1	0	0	7	2
Syn. Sc.3513	0	0	0	7	1
Rambler	0	2	0	12	3
Paramount	1	7	0	40	10
Ladak	1	5	1	50	10
Grimm	0	0	0	73	9
Rhizoma	3	3	1	42	10
Vernal	1	3	1	49	9
Sevelra No. 3	3	11	3	57	16
Ranger	5	8	5	61	18
Du Puits	73	0	0	88	84
Lahontan	94	0	0	88	105
Australian Common	97	0	0	85	107

*Winter injury index was calculated on the basis of:
Total % W.I. 1955-56 + ½% W.I. 1956-57 + ¼% W.I. 1957-58 + ⅛% W.I. 1958-59.

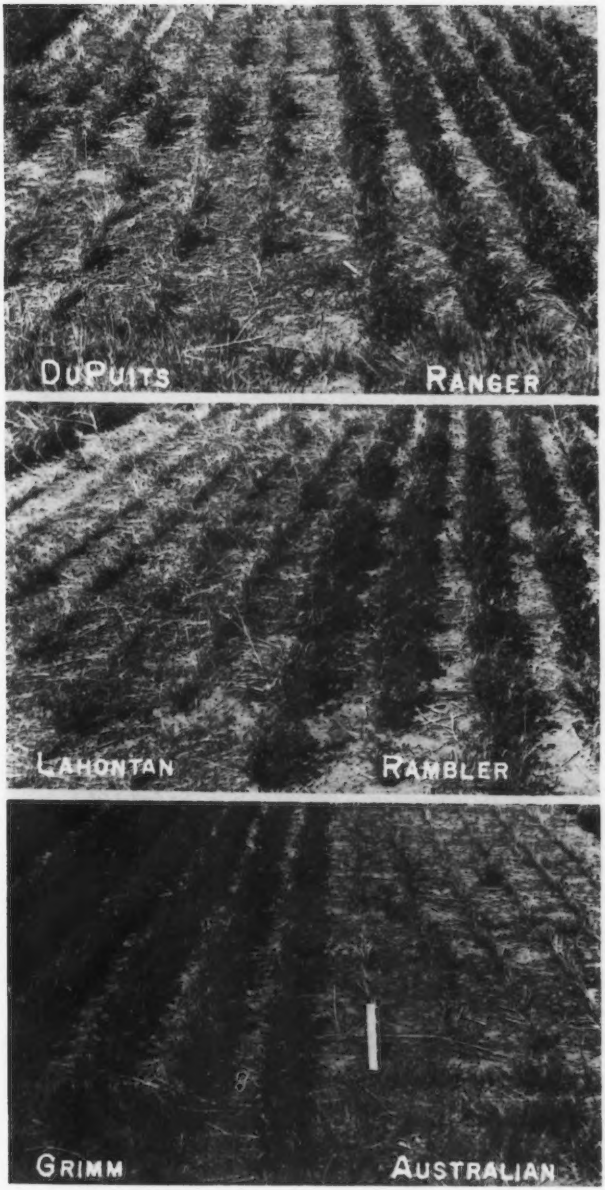

FIGURE 1. Differential winterkilling of alfalfa varieties during the first winter. Grimm, Rambler and Ranger suffered no reduction of stand, while Australian, Lahontan and Du Puits were practically eliminated. (Photos taken May 30, 1956).

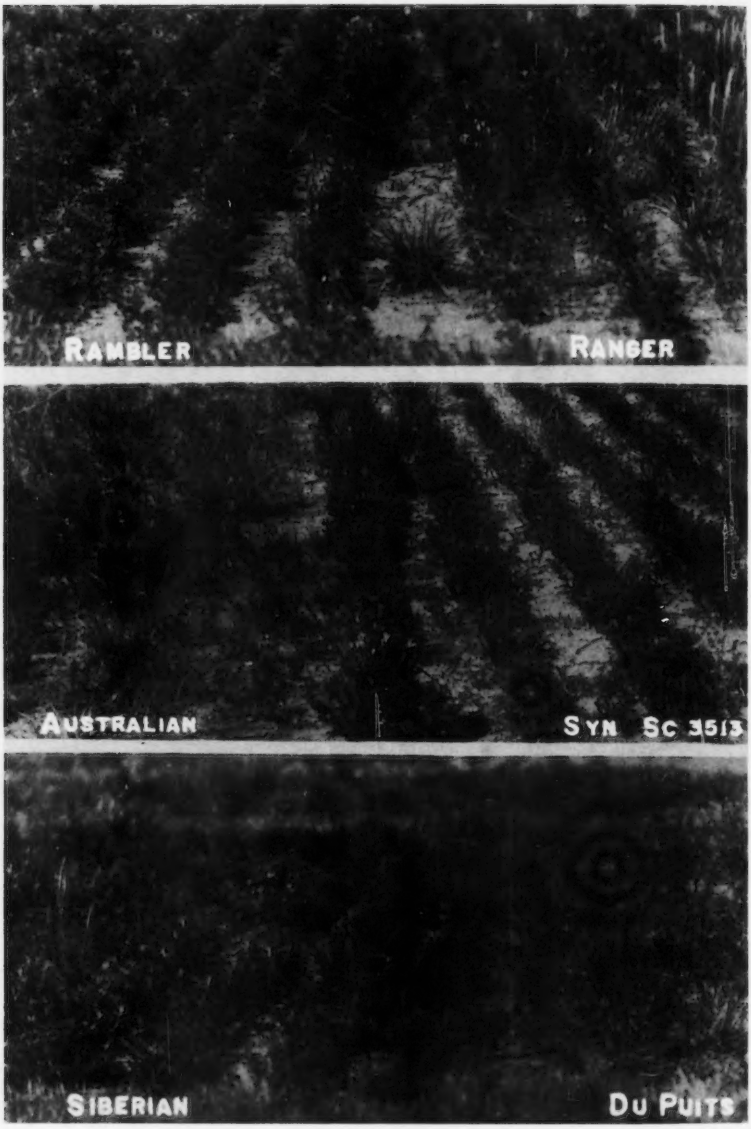

FIGURE 2. The relative stand of several alfalfa varieties after the fourth winter. Siberian, Syn Sc. 3513 and Rambler were still vigorous and little grass invasion was in evidence. Ranger, although still present in complete rows, had allowed considerable invasion of grass because of the winter injury which it suffered. The plots of Du Puits and Australian contained only a few alfalfa plants and many invading grasses. (Photos taken June 4, 1959).

A study of S-1-N derivatives of known mixtures of glucose and xylose gave the results shown in Table 1. The significance of these regressions indicates that at the wavelengths studied the S-1-N derivatives of hexoses and pentoses exhibited "additive absorbances" (14). Consequently, the "algebraic method for additive absorbances" for a "two component system" (14) could be applied.

The two wavelengths offering the most accurate estimates of hexoses and pentoses were determined by the method of Berry (1) and were found to be 580 and 530 mu. Accordingly the absorbances of the S-1-N derivatives of the alfalfa root extracts (2) were measured at these two wavelengths and the concentrations of hexoses and pentoses calculated as described by Stearns (14).

The data on winter injury throughout the period of the test are presented in Table 2. During the first winter, Lahontan and Australian were injured to the extent of over 95 per cent while Du Puits suffered 73 per cent injury; actually the winter injury was so severe in these varieties that most of the affected plants were killed completely. Other varieties were not injured appreciably (Figure 1). During the next 2 years there was some injury to several varieties but the remaining plants of the three non-hardy varieties did not exhibit any damage whatsoever. These plants, of course, were growing under less crowded conditions and individual plants were larger, which may have contributed to their greater vigour and good survival rate. Another reason might be that the surviving plants within these varieties were inherently hardier than the norm of the variety. Very severe winter injury, but little complete killing, occurred during the winter of 1958-59 which followed an extremely dry fall. All but three varieties suffered severe injury while the plants of Du Puits, Lahontan and Australian, which had survived the previous years, were practically all killed out (Figure 2). The winter injury index given in the last column of Table 2 shows the relative winter hardiness of the 13 varieties based on a weighted system whereby winter injury suffered the first season is given a weight of 1, the second year ½, the third year ¼, and the fourth year ⅛. This, in the opinion of the writers, is a fair system of evaluating the varieties for winter hardiness since the longer a variety survives the hardier it must be. The classification of the varieties for winter hardiness according to this test comes very close to that given by Heinrichs (11) which is based on observations in numerous field tests. The correlation coefficient between Heinrichs' rating and the winter injury calculated according to this test is +.88.

In Table 3 data for certain varietal characteristics are presented. It is evident that the yield of the varieties was very low over the 3-year period reflecting the extremely dry conditions under which the experiment was conducted.

The varieties differed widely in recovery during the first 2 weeks in October of the first season. The low figures indicate quick recovery and the high ones slow recovery. The root development in the top 6 inches of soil during the establishment year differed significantly between varieties.

TABLE 3.—VARIABILITY BETWEEN CERTAIN CHARACTERS OF ALFALFA VARIETIES
6-replicate test seeded May 1955

Variety	Seedling year — 1955		% Soluble carbohydrates of root D.M. in top 6 inches of soil				% D.M. tons/acre 56-58 average
	Recovery 1-5*** Oct. 1-18/55	Root weight lb./acre Oct. 15/55	Oct. 15/55		Oct. 15/58		
			Hexosans	Pentosans	Hexosans	Pentosans	
Siberian — Sask.	4.3	1329	43.7	2.9	22.2	3.7	.44
Syn. Sc.3513	5.0	1118	42.3	2.8	81.0	3.5	.44
Rambler	4.0	1305	44.6	3.5	20.4	3.6	.53
Paramount	3.3	1727	44.7	2.9	18.9	3.9	.44
Ladak	2.7	1569	47.6	2.9	21.8	3.4	.52
Grimm	2.2	1535	46.9	2.9	20.5	3.8	.48
Rhizoma	2.7	1621	46.2	2.9	21.7	3.6	.47
Vernal	2.5	1669	49.4	3.1	22.9	3.9	.55
Sevelra No. 3	2.5	1640	47.8	2.4	23.4	3.6	.45
Ranger	2.0	1693	49.2	2.2	23.8	3.9	.43
Du Puits	1.2	1593	48.7	2.7	22.3	3.5	—
Lahontan	1.0	1837	56.9	2.5	24.5	3.8	—
Australian Common	1.0	1737	51.7	2.6	23.6	3.4	—
Significant	**	**	**	N.S.	**	N.S.	**
S.E. mean	±.14	±174	±1.1	±.3	±.9	±.2	±.02
C.V. of expt. %	13.8	16.5	5.6	26.9	10.6	11.2	19.0

*Significant at 5% point
**Significant at 1% point
***Recovery rated 1 to 5, where 1 represents rapid recovery and 5 very slow recovery

TABLE 4.—RELATIONSHIP OF CERTAIN CHARACTERS WITH WINTER INJURY AND INTERRELATIONSHIP OF SEVERAL OF THESE CHARACTERS

Winter injury indices correlated with:	r Value***
Recovery between Oct. 1 and Oct. 18 Seedling year	−.79**
Root weight in top soil — Oct. 15 Seedling year	+.55*
Hexosans — Oct. 15/55 Seedling year	+.80**
Pentosans — Oct. 15/55 Seedling year	−.42
Hexosans — Oct. 15/58 3rd crop year	+.56*
Pentosans — Oct. 15/58 3rd crop year	−.23
Recovery correlated with:	
Root weight first fall	−.84**
Hexosans first fall	−.87**
Hexosans fourth fall	−.74**

*Significant at P = .05
**Significant at P = .01
***r values between recovery and other characters appear negative (−) because of the method of rating recovery (Table 3) although actually the relationship is positive according to the stated comparison.

The trend was for the hardier varieties to develop a smaller quantity of roots in the first season than the less hardy ones.

The amount of hexosans in the roots differed significantly between varieties but not the pentosans. The hardy varieties contained a lower proportion of hexosans than the non-hardy ones in the root material. These differences were more apparent in 1955 on the seedling plants than in 1958 on 3½ year-old plants. In 3 years there had been very severe winter killing in the non-hardy varieties so that the analysed roots perhaps were from hardy surviving plants, thus bringing about a narrower differential between remaining plants of the hardy and non-hardy alfalfa varieties.

The associations between various characters and winter injury are shown in Table 4 along with relationships between recovery and several of the characters. Recovery was closely associated with winter injury, the negative correlation coefficient of −.79 meaning that quick recovery types of alfalfa suffer more winter damage than slow recovery types. Root weight in the first season was significantly correlated with winter injury, the varieties which developed less roots being the hardier ones.

The hexosan content in the roots in October of the first season was closely associated with winter injury, lower hexosan content indicating greater hardiness. A similar trend was apparent for the hexosan content in the fall of the third crop year, but the association was of a lower order. This lower correlation could be expected since, in the non-hardy varieties, only the hardy plants remained by the third year. The pentosans were not significantly correlated with winter injury but the association tended to be in the negative direction. For this reason it would appear to be best to use only hexosans rather than total soluble carbohydrates as an indication of hardiness qualities in alfalfa.

Recovery was shown to be closely associated with the hexosan content and root weight.

The results indicate that a combination of the recovery characteristic and hexosan content in the roots could be used in screening alfalfa breeding material for hardiness qualities. Thus, selections made from F_2 populations originating from hybrids of hardy and non-hardy stocks could be analysed for hexosan content when dug-up in the fall; the roots of each plant could be used for this purpose while stem cuttings could serve the propagation requirements.

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THE USE OF DIALLEL CORRELATIONS FOR DETERMINING THE RELATIVE LOCATIONAL PERFORMANCE OF VARIETIES OF BARLEY

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ABSTRACT

Using diallel correlation coefficients based on the yield of nine varieties of barley, eight of the ten test locations in the Upper Peace River region can be arranged into three completely inter-related response groups. Each group has one location in common with the adjoining group. The locational associations for height, bushel weight and 1000-kernel weight are not as consistent but, with a few exceptions, approximate those established for yield. The groupings suggest that the number of barley test locations in the region could be reduced from ten to five without an appreciable loss of information.

INTRODUCTION

Because of locational differences in crop development, supplementary testing is considered necessary in most regions for the final evaluation of new cereal varieties and hybrids. However, varieties that are sufficiently well adapted at one location to enter the supplementary tests should respond similarly at other locations within a broad soil-climatic region. If so, it should be possible to arrange existing test locations into several response groups and to systematically reduce the number by using only one representative location from each group.

Grouping of locations on the basis of the yield of a number of varieties has been attempted for oats (1) and corn (2) using the size of the variety x location interaction as the criterion of similarity of locational performance. However, diallel correlations, whereby the performance of a number of varieties at one location is correlated with the performance at each of the other locations, provides a more direct method of determining locational relationships.

The present study is designed to evaluate diallel correlations for measuring the relative performance of nine varieties of barley at ten locations in the Upper Peace River region.

MATERIALS AND METHODS

The relative performance of nine varieties of barley at Beaverlodge, Blueberry Mountain, Fairview, High Prairie, North Pine and Wanham on Black to Degraded Black soils and at Baldonnel, Goodfare, Hines Creek and McLennan on Grey Wooded soil was determined by calculating correlation coefficients for all pairs of locations. Performance was based on yield in bushels per acre, height in inches, weight per bushel in pounds and 1000-kernel weight in grams of Edda, Edda II, Gateway, Husky, Olli, Vantage, Vantmore, Wolfe and Br. 3902 barley. Three years' data, 1954-56, were averaged for each characteristic at all locations except Blueberry Mountain, Hines Creek and McLennan for which only two years' data were available. All coefficients were calculated to three decimal places but were contracted to one decimal place in the tables.

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TABLE 1. — CORRELATION COEFFICIENTS FOR YIELD OF NINE VARIETIES OF BARLEY GROWN AT TEN LOCATIONS DURING 1954-56, INCLUSIVE

Location	North Pine	High Prairie	Hines Creek	Mc-Lennan	Good-fare	Beaver-lodge	Fair-view	Wan-ham	Blueberry Mountain	Bal-donnel
North Pine	—	.8	.7	.6	.4	.5	.4	.0	.2	.5
High Prairie	.8	—	.8	.7	.6	.6	.5	.1	.0	.4
Hines Creek	.7	.8	—	.9	.7	.8	.6	.4	.1	.4
McLennan	.6	.7	.9	—	.8	.8	.5	.5	.1	.1
Goodfare	.4	.6	.7	.8	—	.9	.7	.6	.5	.3
Beaverlodge	.5	.6	.8	.8	.9	—	.8	.7	.5	.4
Fairview	.4	.5	.6	.5	.7	.8	—	.7	.6	.4
Wanham	.0	.1	.4	.5	.6	.7	.7	—	.3	.0
Blueberry Mountain	.2	.0	.1	.1	.5	.5	.6	.3	—	.6
Baldonnel	.5	.4	.4	.1	.3	.4	.4	.0	.6	—

Note: Correlation coefficients of .7 exceed the P. 05 level of significance

TABLE 2. — CORRELATION COEFFICIENTS FOR HEIGHT OF NINE VARIETIES OF BARLEY GROWN AT TEN LOCATIONS DURING 1954-56, INCLUSIVE

Location	North Pine	High Prairie	Hines Creek	Mc-Lennan	Good-fare	Beaver-lodge	Fair-view	Wan-ham	Blueberry Mountain	Bal-donnel
North Pine	—	.9	.7	.5	.7	.6	.7	.7	.5	.9
High Prairie	.9	—	.7	.6	.8	.8	.6	.7	.5	.8
Hines Creek	.7	.7	—	.2	.5	.3	.4	.4	.4	.7
McLennan	.5	.6	.2	—	.7	.6	.8	.7	.3	.6
Goodfare	.7	.8	.5	.7	—	.8	.8	.6	.7	.8
Beaverlodge	.6	.8	.3	.6	.8	—	.5	.7	.0	.6
Fairview	.7	.6	.4	.8	.8	.5	—	.7	.7	.8
Wanham	.7	.7	.4	.7	.6	.7	.7	—	.2	.8
Blueberry Mountain	.5	.5	.4	.3	.7	.0	.7	.2	—	.7
Baldonnel	.9	.8	.7	.6	.8	.6	.8	.8	.7	—

Note: Correlation coefficients of .7 exceed the P. 05 level of significance

RESULTS

Yield

Based on the correlation coefficients presented in Table 1, eight of the locations can be placed in three completely inter-related groups, each group having one location in common with the adjoining group. For easy reference, North Pine, High Prairie and Hines Creek are referred to as *Group I;* Hines Creek, McLennan, Goodfare and Beaverlodge as *Group II*, and Beaverlodge, Fairview and Wanham as *Group III*. The precision of the grouping is indicated by the fact that the average correlation coefficients for Groups I ($\bar{r} = 0.754$), II ($\bar{r} = 0.828$) and III ($\bar{r} = 0.715$) are much higher than the average for the ungrouped associations ($\bar{r} = 0.401$). The two aberrant significant correlations, McLennan with High Prairie and Fairview with Goodfare, tend to strengthen the associations between Groups I and II, and Groups II and III, respectively. The yield responses at Blueberry Mountain and Baldonnel are not associated with those at any of the other locations.

Height

The correlation coefficients for height can be manipulated to form several completely or partially inter-related groups of locations. However, the grouping is not as precise as that established for yield. Rather than attempt to establish new relationships, Table 2 is constructed to indicate how the grouping established for yield applies to the height associations. For Group I the fit is satisfactory ($\bar{r} = 0.771$) but for Group II ($\bar{r} = 0.519$) and Group III ($\bar{r} = 0.653$) the fit is inadequate compared with the average correlation for the ungrouped associations ($\bar{r} = 0.614$).

Bushel Weight

Higher correlation coefficients and a broader grouping of locations indicate that bushel weight is a more stable varietal characteristic than yield or height but, as shown in Table 3, the grouping is not as precise as for yield. However, the average correlation for the ungrouped associations ($\bar{r} = 0.682$) is appreciably lower than for Groups I ($\bar{r} = 0.806$), II ($\bar{r} = 0.771$) and III ($\bar{r} = 0.820$) indicating an adequate fit to the grouping of locations established for yield.

1000-Kernel Weight

The correlation coefficients presented in Table 4 show that the 1000-kernel weight response was significantly correlated for all locations. Although kernel weight appears to be a very stable characteristic, there were some variations in the degree of association of different locations. The averages of the correlation coefficients for Groups I ($\bar{r} = 0.959$) and II ($\bar{r} = 0.932$) are slightly higher than for the ungrouped associations ($\bar{r} = 0.865$) suggesting a grouping similar to yield. The fit is not satisfactory for Group III ($\bar{r} = 0.769$).

DISCUSSION

The use of diallel correlations provides a satisfactory method of grouping the ten test locations for barley in the Upper Peace River region into response groups. Yield is the most suitable characteristic for deriving

TABLE 3. — CORRELATION COEFFICIENTS FOR BUSHEL WEIGHT OF NINE VARIETIES OF BARLEY GROWN AT TEN LOCATIONS DURING 1954-56, INCLUSIVE

Location	North Pine	High Prairie	Hines Creek	Mc-Lennan	Good-fare	Beaver-lodge	Fair-view	Wan-ham	Blueberry Mountain	Bal-donnel
North Pine	—	.7	.9	.9	.8	.8	.6	.8	.3	.6
High Prairie	.7	—	.8	.6	.7	.7	.8	.9	.8	.8
Hines Creek	.9	.8	—	.9	.8	.8	.8	.9	.6	.8
McLennan	.9	.6	.9	—	.7	.7	.7	.8	.3	.8
Goodfare	.8	.7	.8	.7	—	.7	.5	.5	.3	.6
Beaverlodge	.8	.7	.8	.7	.7	—	.8	.8	.4	.8
Fairview	.6	.8	.8	.7	.5	.8	—	.8	.7	.9
Wanham	.8	.9	.9	.8	.5	.8	.8	—	.7	.9
Blueberry Mountain	.3	.8	.6	.3	.3	.4	.7	.7	—	.6
Baldonnel	.6	.8	.8	.8	.6	.8	.9	.9	.6	—

Note: Correlation coefficients of .7 exceed the P. 05 level of significance

TABLE 4. — CORRELATION COEFFICIENTS FOR 1000-KERNEL WEIGHT OF NINE VARIETIES OF BARLEY GROWN AT TEN LOCATIONS DURING 1954-56, INCLUSIVE

Location	North Pine	High Prairie	Hines Creek	Mc-Lennan	Good-fare	Beaver-lodge	Fair-view	Wan-ham	Blueberry Mountain	Bal-donnel
North Pine	—	.9	.9	.9	.9	.8	.8	.8	.8	.9
High Prairie	.9	—	.9	.9	.9	.9	.9	.8	.9	.9
Hines Creek	.9	.9	—	.9	.9	.9	.9	.8	.9	.9
McLennan	.9	.9	.9	—	.9	.9	.8	.7	.8	.9
Goodfare	.9	.9	.9	.9	—	.9	.9	.8	.9	.9
Beaverlodge	.8	.9	.9	.9	.9	—	.7	.7	.8	.9
Fairview	.8	.9	.9	.8	.9	.7	—	.9	.9	.9
Wanham	.8	.8	.8	.7	.8	.7	.9	—	.8	.8
Blueberry Mountain	.8	.9	.9	.8	.9	.8	.9	.8	—	.9
Baldonnel	.9	.9	.9	.9	.9	.9	.9	.8	.9	—

Note: Correlation coefficients of .7 exceed the P. 05 level of significance

a precise grouping of locations. The locational associations for height, bushel weight and 1000-kernel weight are not as precise but, with a few exceptions, approximate those established for yield.

The relationships are not based on the geographical proximity of locations and do not appear to be defined by recognized soil or climatic characteristics. However, the precise grouping of locations and the interlocking of the response groups indicate that the associations are caused by a linear variation of one or more environmental characteristics.

The groupings suggest that the number of test locations in the Upper Peace River region can be reduced from ten to five without an appreciable loss of information by testing at only one of the locations in each of the three response groups and at Baldonnel and Blueberry Mountain. The associations are based on an average of 3 years' data for all but three locations, essentially obscuring the influence of yearly variations in climate. Also, the nine varieties should be sufficiently diverse genotypically to assess the response of most adapted varieties. However, the application of this grouping must be confirmed by further testing, preferably with another group of barley varieties.

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ECONOMIC LOSSES CAUSED BY WEED COMPETITION IN MANITOBA GRAIN FIELDS

II. EFFECT OF WEED COMPETITION ON THE PROTEIN CONTENT OF CEREAL CROPS[1]

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ABSTRACT

Economic losses caused by weed competition in Manitoba grain crops were studied on 142 farm fields during a 3-year period, 1956 to 1958 inclusive. Ten paired plots were located in each farm field, one-half of each paired plot being kept weed-free throughout the growing season. Per cent protein was determined on wheat, barley and oat samples collected from 60 of these farm fields. Significant increases in protein content were obtained following weed removal in 22 of the 60 grain fields studied and small increases were noted in most other fields. In the combined analysis of all fields, removal of weeds resulted in significant increases ($P = .01$) in both protein content and yield of wheat, barley and oats. This suggests that weeds compete very effectively with grain crops for available nitrogen and that reductions in crop yields resulting from weed competition are generally accompanied by reductions in protein content as well.

INTRODUCTION

High crop yields have often been associated with a low protein content of harvested grain and thus any agronomic practice that tends to raise yield could be suspected of lowering the protein content. Certainly one such practice would be the control of weeds either by cultural methods or by herbicidal sprays.

High crop yields resulting from better than average climatic conditions and good agronomic practices have resulted in decreases in the protein content of harvested grain. For example, when water supplies are high, plants produce more vegetative growth, thus using much of the available nitrogen for vegetative parts (11). Increases in yield resulting from nitrogen fertilization generally do not result in increases in protein content, probably because the added nitrogen has been entirely utilized earlier in the season for vegetative growth (7, 9, 10). Applications of 2,4-D at recommended dosages to cereal crops have often resulted in reduced weed competition with attendant increases in yield (1, 3, 5) and several investigators have reported that treatment with 2,4-D, either directly or indirectly, also increased the protein content of wheat (2, 3, 4, 6, 8). Since these observations were obtained from small experimental plots and may not necessarily be indicative of what happens under actual farm field conditions, information concerning the influence of weed control practices on yield and protein content of grain harvested from farm fields is required. This paper deals with an experiment designed to measure these effects under actual farm conditions.

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MATERIALS AND METHODS

Yield and per cent protein of grain from 60 farm fields in Manitoba were determined. The fields sampled were all within a 60-mile radius of Winnipeg. From each field, samples were available from 10 pairs of weed-free and weedy plots. The weed-free portion of each paired plot was hand-weeded at weekly intervals throughout the summer and square yard samples were harvested for yield and protein content determinations. The protein content was determined on samples harvested from 22 wheat fields and 12 barley fields in 1957 and from 11 wheat fields, 9 barley fields, and 6 oat fields in 1958.

The protein content was determined by the conventional macro Kjeldahl procedure using copper sulphate as a catalyst. Each sample was analysed for moisture by the 130°C. oven method and protein results were calculated to a 13.5 per cent moisture level. The factors used for converting per cent nitrogen to per cent protein were 5.7 for wheat and 6.25 for barley. The data were analysed by the paired comparison "t" test method.

RESULTS AND DISCUSSION

Average weed counts, yield and protein content data from the 22 wheat fields and 12 barley fields studied in 1957 are presented in Table 1.

The nature and extent of the weed infestations encountered in the various fields have already been discussed (5). Significant increases in both yield and protein content were obtained as a result of weed removal in 14 of the 34 fields studied and small increases were noted in most of the other fields. In the combined analysis of all fields weed removal resulted in highly significant increases in both yield and protein content of wheat and barley.

Similar results were obtained in 1958. Average weed counts, yield and protein content data are presented in Table 2. Significant increases in both yield and protein content were obtained in only 6 of the 26 fields studied when weeds were removed but small increases were again noted in most of the other fields. The average increase in both yield and protein content resulting from weed removal for the 11 wheat fields was significant at $P = .01$. Similarly, significant increases ($P = .05$) for both yield and protein content were obtained for the average of the 9 barley fields and 6 oat fields studied.

On the basis of results obtained over the 2-year period it was noted that when yields of grain were increased by weed removal, protein content also was increased. This suggests that weeds compete very effectively with the crop for available nitrogen to the point that reductions in yield resulting from weed competition are generally accompanied by reductions in protein content as well.

ACKNOWLEDGEMENT

This study was made possible through an extra-mural research grant from the Science Service, Canada Department of Agriculture, Ottawa. This assistance is gratefully acknowledged.

TABLE 1. — EFFECT OF WEED COMPETITION ON THE PROTEIN CONTENT AND YIELD OF WHEAT AND BARLEY, 1957

Location	Previous cropping	Weeds per sq. yd.	Yield of grain (bushels per acre)			Protein content of grain (per cent)		
			Weed-free	Weedy	Difference	Weed-free	Weedy	Difference
				WHEAT				
C-3	fallow	15	34.5	35.8	−1.3	11.02	11.16	−.14
B-3	fallow	36	31.6	30.6	1.0	14.31	13.98	.33*
M-8	fallow	65	32.4	29.7	2.7*	13.10	12.57	.53*
M-13	fallow	67	33.6	33.0	0.6	14.24	14.09	.15
C-8	fallow	77	41.9	37.5	4.4*	11.82	11.83	−.01
B-6	fallow	98	38.2	30.1	8.1**	12.51	12.19	.32*
M-7	fallow	98	37.8	35.7	2.1	15.02	15.25	—.23
M-19	fallow	105	15.8	14.5	1.3*	11.11	10.71	.39**
B-4	fallow	109	39.7	36.6	3.1*	11.26	11.16	.10
M-15	fallow	161	38.0	38.1	—0.1	12.75	12.63	.12
S-1	fallow	164	37.7	32.1	5.6**	14.46	14.33	.13
B-2	fallow	186	26.1	25.3	0.8	10.72	10.64	.08
M-14	fallow	225	29.8	21.3	8.5**	13.59	13.52	.07
S-7	fallow	253	27.9	17.2	10.7**	10.68	10.68	.00
P-4	fallow	275	35.5	32.9	2.6*	12.35	12.13	.22*
M-16	fallow	278	19.9	13.9	6.0**	13.26	13.01	.25*
C-6	fallow	404	46.4	39.4	7.0**	13.11	12.98	.13
P-8	fallow	465	27.8	24.5	3.3*	12.93	12.52	.41*
M-20	stubble	54	11.0	10.7	0.3	11.89	11.64	.25
M-11	stubble	246	20.7	16.2	4.5*	11.61	11.51	.10
M-1	stubble	283	12.8	9.9	2.9*	9.43	9.02	.41*
P-2	stubble	416	16.2	8.0	8.2**	10.45	9.47	.98**
Mean		184	29.8	26.0	3.8**	12.35	12.14	.21**

TABLE 1. — EFFECT OF WEED COMPETITION ON THE PROTEIN CONTENT AND YIELD OF WHEAT AND BARLEY, 1957 — CONTINUED

Location	Previous cropping	Weeds per sq. yd.	Yield of grain (bushels per acre)			Protein content of grain (per cent)		
			Weed-free	Weedy	Difference	Weed-free	Weedy	Difference
				BARLEY				
C-7	stubble	41	33.3	33.0	0.3	9.47	9.29	.19
S-9	stubble	60	24.2	22.7	1.5	9.94	9.67	.27
S-5	stubble	68	26.6	24.9	1.7	10.16	10.46	−.30
M-12	stubble	90	14.9	13.8	1.1	11.08	10.77	.31
P-9	stubble	178	23.5	16.4	7.1**	8.89	8.40	.49*
P-3	stubble	230	42.1	37.3	4.8*	9.47	9.19	.28*
C-1	stubble	356	36.8	27.1	9.7*	9.97	9.42	.55**
M-18	stubble	384	17.7	14.6	3.1*	9.55	9.44	.11*
P-7	stubble	395	39.5	31.3	8.2**	10.96	10.97	—.01
P-1	stubble	568	43.2	34.7	8.5*	10.13	9.70	.43**
C-2	stubble	658	14.8	5.7	9.1**	8.90	8.94	—.04
S-4	stubble	867	14.6	12.4	2.2*	9.98	9.56	.42*
Mean		325	27.6	22.8	4.8**	9.88	9.65	.23**

*Difference significant at the 5% level of probability
**Difference significant at the 1% level of probability

TABLE 2. — THE EFFECT OF WEED COMPETITION ON THE PROTEIN CONTENT AND YIELD OF WHEAT, BARLEY AND OATS, 1958

Location	Previous cropping	Weeds per sq. yd.	Yield of grain (bushels per acre)			Protein content of grain (per cent)		
			Weed-free	Weedy	Difference	Weed-free	Weedy	Difference
				WHEAT				
M-1	fallow	19	23.2	21.9	1.3	12.18	12.91	—.01
M-3	fallow	30	13.0	11.5	1.5	12.18	12.00	.18
M-12	fallow	46	27.2	25.6	1.6	12.33	12.17	.16
P-2	fallow	74	20.3	17.3	3.0*	11.30	11.21	.09
M-13	fallow	81	24.4	21.4	3.0	15.31	15.06	.25
M-18	fallow	93	21.6	17.9	3.7*	11.94	11.81	.13
B-2	fallow	142	39.3	36.8	2.5	14.86	13.91	.95**
M-6	fallow	146	27.6	24.1	3.5*	14.15	13.74	.41*
M-10	fallow	178	27.2	23.6	3.6*	13.56	13.20	.36*
M-7	stubble	28	41.8	40.8	1.0	14.82	14.80	.02
M-11	stubble	113	17.4	15.5	1.9*	14.06	13.35	.71*
Mean		86	25.7	23.3	2.4**	13.34	13.04	.30*

TABLE 2. — THE EFFECT OF WEED COMPETITION ON THE PROTEIN CONTENT AND YIELD OF WHEAT, BARLEY AND OATS, 1958 — CONTINUED

Location	Previous cropping	Weeds per sq. yd.	Yield of grain (bushels per acre)			Protein content of grain (per cent)		
			Weed-free	Weedy	Difference	Weed-free	Weedy	Difference
				BARLEY				
B-3	stubble	11	55.5	50.2	5.3	10.66	10.36	.30
S-8	stubble	40	23.1	20.2	2.9	12.08	11.96	.12
M-15	stubble	63	33.3	30.2	3.1	11.28	11.20	.08
C-8	stubble	99	26.7	27.0	−0.3	9.98	9.89	.09
M-19	stubble	105	18.6	14.5	4.1*	12.47	11.91	.56*
P-5	stubble	212	45.1	45.8	−0.7	11.19	10.96	.23
M-14	stubble	308	36.8	30.9	5.9*	11.75	11.51	.24
S-4	stubble	340	20.2	19.9	0.3	10.75	10.09	.66*
P-7	fallow	542	59.7	56.6	3.1	12.86	13.03	—.17
Mean		191	35.4	32.8	2.6*	11.45	11.21	.24*
				OATS				
S-3	stubble	17	71.9	63.9	8.0	11.60	11.43	.17
B-4	stubble	39	57.3	53.9	3.4	9.88	9.85	.03
P-9	stubble	84	36.5	27.2	9.3*	10.80	10.37	.43*
M-16	stubble	105	43.5	36.3	7.2*	10.12	9.64	.48*
S-2	stubble	157	47.4	47.6	−0.2	9.75	9.49	.26
C-6	stubble	773	52.6	44.5	8.1*	11.01	10.93	.08
Mean		196	51.5	45.6	5.9*	10.53	10.28	.24*

*Difference significant at 5% level of probability
**Difference significant at 1% level of probability

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FURTHER STUDIES OF MERCURY RESIDUES ON APPLE FRUIT[1]

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ABSTRACT

Following single cover sprays of Erad (phenyl mercury acetate) mercury residues in apples declined until early in August and then increased until at harvest they were greater than the initial deposits. The accumulation of mercury in the maturing fruit appeared to be by translocation from other parts of the tree.

Mylar film was more effective than polythene for protecting fruit from contamination with Erad during and following spraying.

INTRODUCTION

In a previous paper Ross and Stewart (5) reported on the mercury residues of apples at harvest in relation to date of application of organo-mercury cover sprays. They found that a spray of Erad (phenyl mercury acetate) applied on June 17, 25, or July 3 resulted in almost identical harvest residues of about 0.05 p.p.m. even though the surface area of the apples increased four times between the first and last spray. This suggested that, since the calculated initial surface residue was not large enough to account for the total harvest residue, mercury from other parts of the tree accumulated in the fruit.

Other workers (3, 4, 6) have reported harvest mercury residues similar in magnitude to those found by Ross and Stewart (5) and recently Beidas and Higgons (1) found residues ranging from 0.01 to 0.1 p.p.m. on apples from commercial orchards receiving from 5 to 10 applications of mercury fungicides.

Experiments designed to investigate the possibility of mercury being translocated to the fruit from other parts of the tree were carried out during 1959. In addition the relative effectiveness of two plastic films for protecting fruit during and following spraying was investigated. Results obtained are presented in the present paper.

MATERIALS AND METHODS

The same randomized block design of three replicates of 3 Cortland trees per replicate was used as in the previous work (5). Erad[2] at a half-pint per 100 gallons was applied to a tree in each replicate on June 19, 26 and July 7. The diameter of the fruit at each spray date was determined from measurements made on random samples of 50 apples. Before applying the sprays on June 26 and July 7, 100 apples on each tree were enclosed in small polythene bags (0.002-inch thickness). A daub of petrolatum was placed on the stem of the apple around which the neck of the bag was tied tightly with string. When the spray had dried, 50 of the bags from each tree were removed and the apple tagged. Fruit samples for analysis were collected immediately after the spray had dried and at intervals throughout the growing season.

An experiment was also carried out to compare the accumulation of mercury in fruit when covered with bags of polythene and Mylar[3] (Type

[1]Contribution No. 1037 from the Research Station, Canada Department of Agriculture, Kentville, N.S.

[2]Phenyl mercury acetate, 10%. (Green Cross Insecticides, Montreal, Que.).

[3]DuPont Co. of Canada Ltd., Montreal, Que.

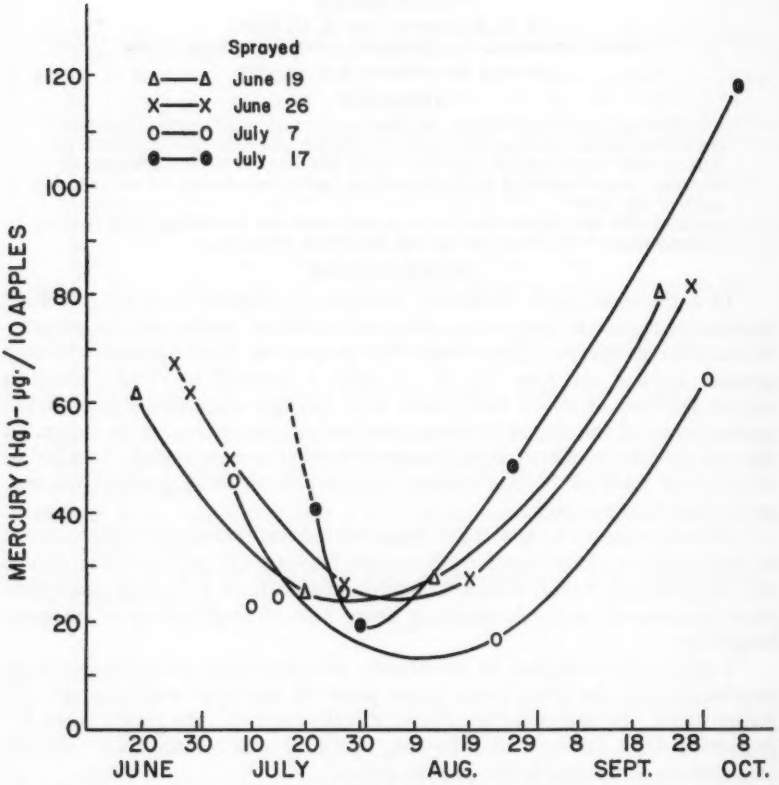

FIGURE 1. Mercury content of apples harvested at intervals between spraying and maturity.

C, 0.001-inch thickness). On July 17, 50 apples on each of 2 Cortland trees were bagged with polythene bags and 50 with Mylar bags as described above. The trees were sprayed with Erad at a half-pint per 100 gallons and immediately after the spray had dried 20 bags of each plastic were removed from each tree and the apples tagged. Samples for analysis were taken at this time and at intervals thereafter.

Ten apples were used for each determination except early in the season, when the apples were small, the sample size varied from 15 to 35. When small apples were analysed the entire fruit was digested but when the apples had reached a suitable size separate analyses were made of peel, pulp, and seeds. In the latter part of July the distribution of mercury in some samples of small apples was determined by removing the calyx end and making separate analyses of the two portions. Spray may more readily adhere to the calyx end of the apple. The calyx ends constituted about 10 per cent of the weight of the samples.

TABLE 1. — DISTRIBUTION OF MERCURY IN SMALL APPLES (MEAN VALUES FOR 3 SAMPLES OF 10 APPLES)

Sprayed	Sampled	Total residue μg. Hg	Calyx residue μg. Hg	Calyx residue as per cent of total residue
June 19	July 20	25.4	10.0	39
June 26	July 27	26.9	16.7	62
July 7	July 27	25.6	16.0	63

TABLE 2. — DISTRIBUTION OF MERCURY RESIDUES IN APPLES (MEAN VALUES FOR 3 SAMPLES OF 10 APPLES)

Sprayed	Sampled	Residue (μg. Hg)			
		Peel	Pulp	Seed	Total
June 19	Aug. 13	3.2	20.9	4.1	28.2
	Sept. 23	27.5	50.8	2.3	80.6
June 26	Aug. 19	6.2	19.1	2.1	27.4
	Sept. 29	32.7	47.2	2.3	82.2
July 7	Aug. 24	1.9	12.6	2.3	16.8
	Oct. 2	23.5	39.0	2.0	64.5
July 17	Aug. 27	12.2	35.4	1.6	49.2
	Oct. 8	50.9	64.9	2.6	118.4

The analytical methods were the same as in previous work (5), except that a 1000-ml. digestion flask was substituted for the 500-ml. flask recommended by Klein (2). This permitted the digestion of undried samples without undue difficulty from foaming.

RESULTS

The mean values for the total mercury in apples at various intervals following sprays applied on June 19, 26, and July 7, at which time the average diameters of the fruit were 11, 15 and 24 mm., respectively, are shown in Figure 1. Those from the Cortland trees sprayed on July 17 are included. The curves for the four spray dates are very similar. On a fresh fruit basis the average total harvest residues of apples sprayed on June 19, 26, July 7, and July 17 were 0.05, 0.05, 0.03, and 0.08 p.p.m., respectively.

The results on the distribution of mercury between the calyx end and the remainder of the fruit are presented in Table 1. They show that in late July a large percentage of the total residue was in the calyx end. The distribution of mercury in peel, pulp, and seeds is shown in Table 2. At harvest the average residue in the peel, pulp, and seeds for the four spray dates was 38, 59, and 3 per cent, respectively, of the total residue.

TABLE 3. — MERCURY CONTENT AT HARVEST OF APPLES BAGGED AND NOT BAGGED AT SPRAYING

Sprayed	Samples	Residue (μg. Hg)			
		Peel	Pulp	Seed	Total
July 7	Bagged	14.5	50.2	—	64.7
	Not bagged	23.5	39.0	2.0	64.5
July 17	Bagged	18.7	21.5	3.0	43.2
	Not bagged	50.9	64.9	2.6	118.4

The results of the experiment on spraying fruit bagged with polythene and the one comparing polythene and Mylar are illustrated in Figures 2 and 3, respectively. During the 8- and 10-day sampling periods rainfall was 0.33 inch and 0.85 inch, respectively. The results illustrated in Figure 3 show the relative effectiveness of the two films in keeping the fruit free from mercury.

The results in Table 3 show the mercury content at harvest of the apples from which the bags were removed following sprays applied on July 7 and 17. Too few apples of those tagged on June 26 remained for a representative sample. Results for fruit from the trees sprayed on July 7 are based on a composite sample of 10 apples from the 3 trees in the treatment; those from the trees sprayed on July 17 are the average of 2 samples of 10 apples from each of 2 trees. Data from fruit not bagged are included.

DISCUSSION

The data illustrated in Figure 1 show that, following cover sprays of the organo-mercury fungicide Erad, the mercury content of apples declined over a period of about a month to approximately one-third of the initial residue. At this time a large part of the residue was concentrated in a small portion of the apple at the calyx end (Table 1). This was followed by a rapid accumulation of mercury in the fruit throughout the remainder of the growing season until at harvest the residues were greater than the initial deposits. The harvest fruit residues due to sprays applied on July 17 were much greater than those from sprays applied on June 19, 26, and July 7 although in early August they had all reached approximately the same minimum value (Figure 1). For all sprays mercury began to accumulate in the fruit at about the same time in August which suggests that the accumulation was influenced by the physiology of the fruit rather than by the time at which the fungicide was applied.

The initial deposits from the sprays applied on June 19 and 26 were somewhat larger than those calculated in 1958 (5), whereas for the July 7 spray they were almost identical. In 1958 it was assumed that, on an area basis, initial leaf and fruit residues were of comparable magnitude. Apparently, at the time the first two sprays were applied, more mercury was deposited on a comparable area of young fruit than on foliage. The initial

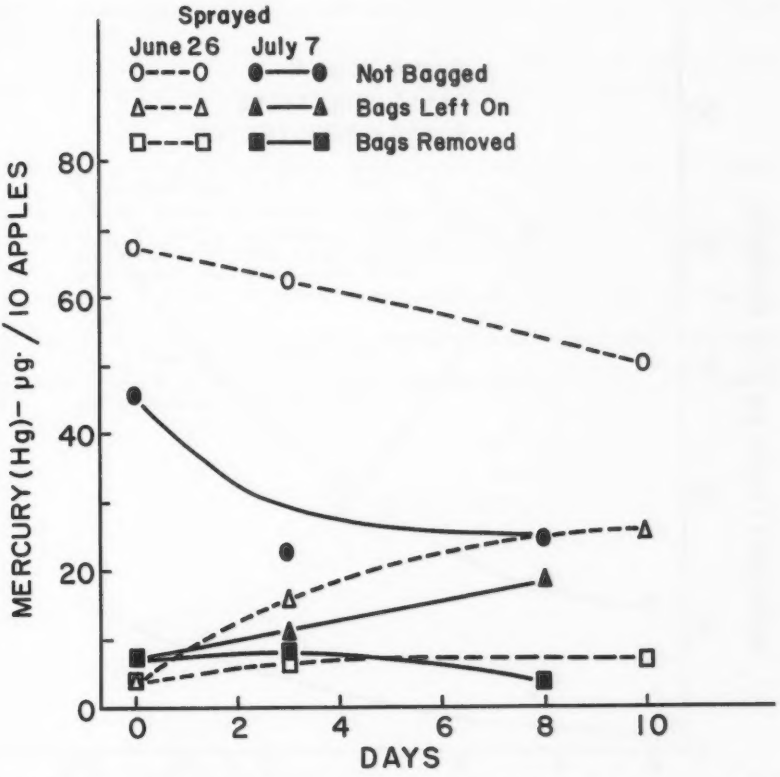

FIGURE 2. Mercury content of apples protected by polythene bags at and following spraying.

deposit per apple from the July 7th spray was much less than that from earlier sprays even though the surface area of the fruit had increased about four times between June 19 and July 7. The disappearance of the fuzz from the apple during this period may explain why less fungicide was able to adhere to its surface.

The results in Table 2 on the distribution of mercury in the fruit show that, as the fruit matured and the total mercury content increased, a larger proportion of the residue was found in the peel whereas the proportion in the pulp decreased. In the August samples, an average of 17 per cent of the total mercury was in the peel. At the later sampling dates this proportion had increased to 38 per cent. The peel comprised about 13 per cent of the total weight of the apple.

Mylar was more effective than polythene in protecting fruit at time of spraying. When fruit remained enclosed in these plastics there was a much more rapid build-up of mercury in the apples bagged with polythene

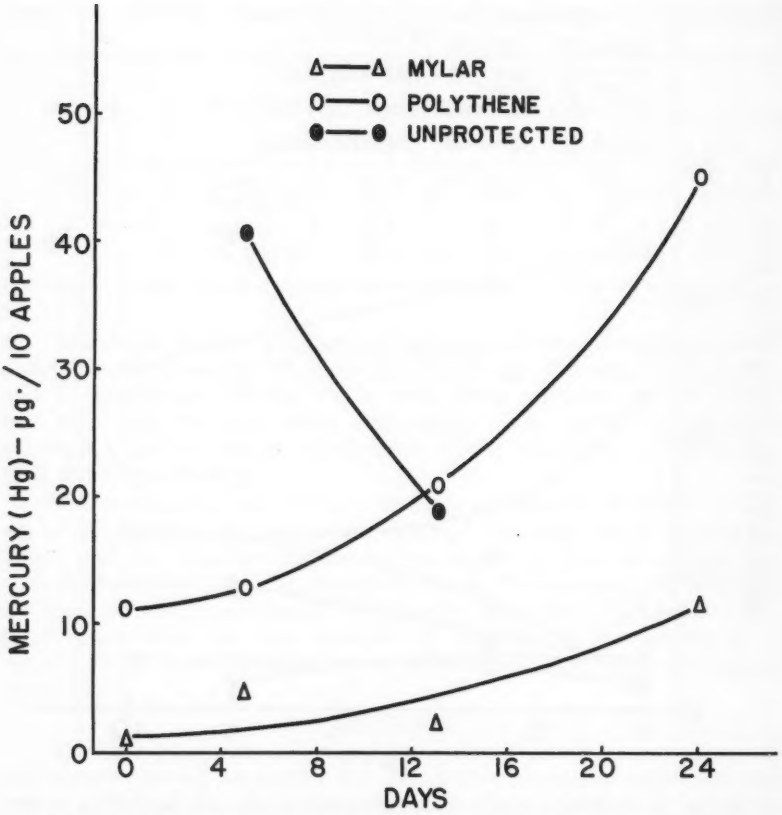

FIGURE 3. Mercury content of apples protected by polythene and Mylar bags at and following spraying.

(Figures 2 and 3). Apparently, polythene, being more permeable, allowed more of the fungicide deposited on the bags to diffuse through to the fruit.

There was no redistribution of mercury to fruit protected at spraying in the 8- to 10-day period following applications of Erad. At harvest these apples contained mercury much in excess of that passing into the bags at spraying. For the July 7th spray harvest residues of a comparable magnitude were found in sprayed and protected fruit although when spray was applied on July 17 larger residues were found in the sprayed apples (Table 3). The distribution of the residue in fruit bagged before spraying was similar to that in unprotected fruit. It has been shown (5) that foliage contains measurable amounts of mercury at least 75 days after a single application of Erad. The mercury which accumulates in the fruit may come from this source. If the build-up of mercury in the fruit was by volatilization or redistribution by weathering there should have been a more

or less gradual accumulation of mercury in the fruit from time of spraying until harvest.

The results of these investigations indicate that as the fruit matured mercury was translocated to the fruit from other parts of the tree.

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THE EFFECT ON GERMINATION OF DRYING LEEK SEED HEADS AT DIFFERENT TEMPERATURES

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ABSTRACT

The effect of drying leek seed heads at various temperatures and temperature combinations upon seed germination was studied from 1951 to 1956. Treatments included 80°F., 95°F., 110°F., and 140°F. continuous, and 80°F. for periods up to 10 days, followed by the higher temperatures. In all seasons a continuous temperature of 80°F. resulted in seed germinating at or above the 65 per cent minimum required by the Canadian Seeds Act, while any treatment including 110°F. or higher caused below-minimum germination. Treatments of 95°F. continuous, and combinations of 80°F. and 90°F., were not significantly different from 80°F. continuous over the period 1953 to 1956, but showed marked trends towards differences in certain years. In 1953 and 1956, germinations from these treatments were well above 65 per cent but below in 1954 and 1955, when summer temperatures were cooler, as shown by heat unit data.

During the 1953-56 period, drying times for the 80°F. continuous treatment averaged 33 days and for 95°F., 21 days. While a continuous temperature of 80°F. was necessary to ensure satisfactory seed germination in all seasons, it is suggested, where rapid drying is desired, that a constant temperature of 95°F. be employed when summer weather favouring maturity has preceded seed harvest.

INTRODUCTION

The growing of leek seed is well suited to the mild climate of southern Vancouver Island where this biennial crop will overwinter successfully without special protection. The seed heads are harvested from late September to mid-October at the beginning of the fall rainy season. As the seed will not fully mature in the field, it is necessary to employ artificial means to dry the seed heads before they can be threshed.

Growers have frequently encountered difficulty in producing seed which will meet the minimum germination of 65 per cent required under the Canadian Seeds Act. An examination of different growing methods by the author (1) failed to reveal any material effect upon seed germination, and the arbitrarily selected drying temperature of continuous 80°F. appeared suitable, as it always resulted in seed which gave germinations above 65 per cent. Inasmuch as growers appear to have paid little attention to the temperature employed during drying of the seed heads, their chief concern being to dry them quickly and economically and then thresh, clean, and ship the seed, this study was initiated to determine the effects of a number of drying temperatures upon seed germination.

MATERIALS AND METHODS

The experiments were conducted during the period 1951-56 with the Musselburgh variety. In each year from 1951 to 1955 seed was sown in the field in early May and seedlings thinned to stand 2 inches apart in rows 3 feet apart. An 8-10-5 fertilizer was broadcast at 1000 pounds per acre in the early spring before tillage in the year of seeding, and over the rows prior to the first cultivation in the second or seed crop year. No irrigation water was applied. The seed heads were cut during the second week of October with 2 inches of stalk attached and a single layer placed in 6- by

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24- by 48-inch seed trays with 14-mesh screen bottoms. The drying rooms measured 8 by 8 by 8 feet and were electrically heated and thermostatically controlled. Forced air circulation was provided to ensure an even temperature throughout the rooms. As the seed heads approached dryness, they were checked twice daily and recorded as "dry" when the pedicels, with seed-bearing capsules attached, separated readily from their point of origin at the apex of the scape. The seed heads were then threshed in a rasp-bar type plot thresher and the seed then cleaned in an Office Clipper cleaner with a No. 7 or No. 8 upper and 1/14 or 1/16 lower screen. The seed and chaff were roughly separated in the first run and the partly cleaned seed then re-run two or three times, each time with slightly increased wind velocity. After hand-picking particles of flower pedicels and receptacles, a representative sample was drawn from each treatment and forwarded to the Plant Products Division, Vancouver, British Columbia, where germination tests were conducted in triplicate.

Drying treatments were as follows:

Year	Initial temperature and duration	Follow-up temperature
1951 and 1952	80°F. continuous	—
	80°F. 10 days (1952 only)	110°F.
	80°F. 8 days (1952 only)	110°F.
	80°F. 6 days	110°F.
	80°F. 4 days	110°F.
	80°F. 2 days	110°F.
	110°F. continuous	—
	80°F. 10 days (1952 only)	140°F.
	80°F. 8 days (1952 only)	140°F.
	80°F. 6 days	140°F
	80°F. 4 days	140°F.
	80°F. 2 days	140°F.
	140°F. continuous	—
1953 to 1956	80°F. continuous	—
	80°F. 10 days	95°F.
	80°F. 8 days	95°F.
	80°F. 6 days	95°F.
	80°F. 4 days	95°F.
	80°F. 2 days	95°F.
	95°F. continuous	—
	80°F. 10 days	110°F.
	80°F. 8 days	110°F.
	80°F. 6 days	110°F.
	80°F. 4 days	110°F.
	80°F. 2 days	110°F.
	110°F. continuous	—

Heat unit data were computed from the daily mean temperatures recorded at the Experimental Farm, Saanichton, B.C., from April 1 to September 30 for the period 1953-56, using a base of 42°F.

RESULTS AND DISCUSSION

1951 and 1952

In both these seasons the only drying treatment to produce seed which germinated about 65 per cent was 80°F. continuous. The 1952 treatment

TABLE 1.—TIME REQUIRED TO DRY SEED HEADS AND PER CENT GERMINATION OF LEEK SEED FOLLOWING VARIOUS DRYING TREATMENTS, 1953-56

Drying treatment	Drying time in days					Per cent germination				
	1953	1954	1955	1956	Mean	1953	1954	1955	1956	Mean
80°F. continuous	26	35	36	34	33	86	65	65	78	74
80°F. 10 days then 95°F.	23	32	27	26	27	91	58	62	81	73
80°F. 8 days then 95°F.	23	31	26	25	26	92	61	47	87	72
80°F. 6 days then 95°F.	22	30	25	24	25	86	59	54	80	70
80°F. 4 days then 95°F.	21	28	24	23	24	87	60	45	77	67
80°F. 2 days then 95°F.	20	26	23	22	23	85	52	44	82	66
95°F. continuous	18	24	22	21	21	90	60	37	75	64
80°F. 10 days then 110°F.	21	24	21	22	22	76	41	20	62	50
80°F. 8 days then 110°F.	21	23	19	21	21	71	40	27	64	50
80°F. 6 days then 110°F.	19	20	18	20	19	72	33	28	63	49
80°F. 4 days then 110°F.	18	18	17	19	18	60	32	8	48	37
80°F. 2 days then 110°F.	17	16	16	18	17	55	24	11	47	34
110°F. continuous	15	14	14	17	15	40	21	2	35	24
Mean	20	25	22	22	—	76	47	35	68	—

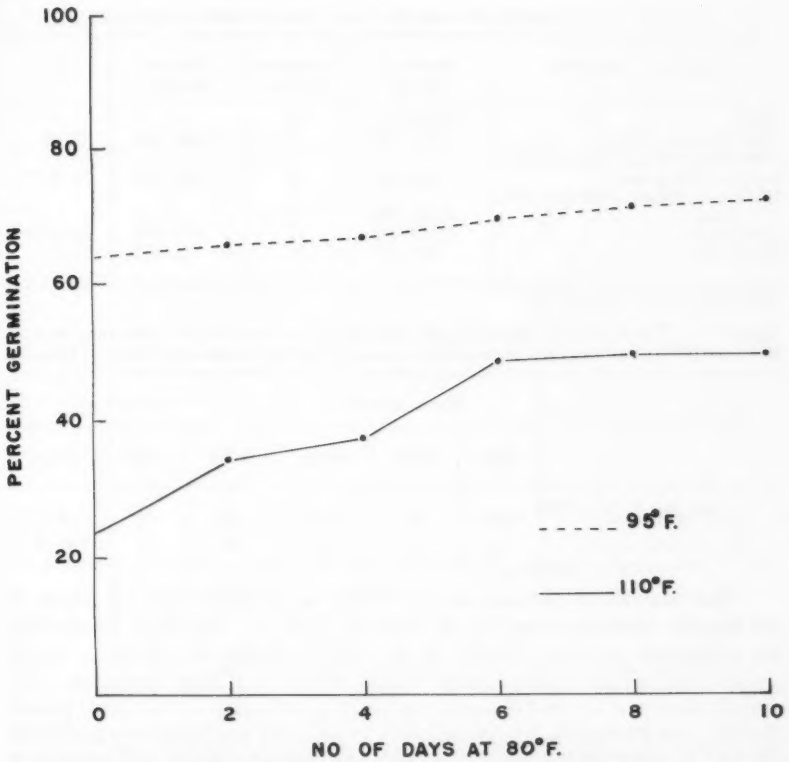

FIGURE 1. The effect of drying leek seed heads for varying initial periods at 80°F., and completed at 95°F. and 110°F., upon seed germination. Data are averaged for the years 1953-56.

of 80°F. for 10 days followed by 110°F. until seed was dry was the second best, yielding seed with 58 per cent germination. It was evident that any treatment involving a temperature of 140°F. was too extreme and this temperature was therefore omitted from further studies.

1953 to 1956

The percentage germination for the years 1953-56 with drying times for each treatment is given in Table 1. These germination data indicate that there may be an important difference between the years 1953 and 1956 on the one hand, and 1954 and 1955 on the other — germinations tending to be higher in the former.

As it is important to the grower that the seed be dried as quickly as possible without impairing its viability, it is noteworthy that there were marked differences between treatments in drying times. In the 95°F. continuous drying treatment, for example, the 4-year average drying time was 12 days less than that for the 80°F. continuous temperature.

TABLE 2. — ANALYSIS OF VARIANCE (DATA TRANSFORMED TO ANGLES)

Sources of variation	Sums of squares	Degrees of freedom	Mean square	F.
Total	12,074.360	51		
Years between groups (1953 and 1956 vs. 1954 and 1955)	5,242.286	1	5,242.286	15.68
Years within groups (1953 and 1956 vs. 1954 and 1955)	668.504	2	334.252	25.81**
Total	5,910.790	3	—	—
Treatments	5,697.432	12	474.786	36.67**
Remainder	466.137	36	12.948	—

**Significant at the 1 per cent level.

TABLE 3. — THE EFFECT OF DRYING LEEK SEED HEADS AT CONTINUOUS TEMPERATURES OF 80°F. AND 95°F. UPON PER CENT SEED GERMINATION AFTER TWO WARM AND TWO COOL SEASONS

Drying treatment	Warm seasons			Cool seasons		
	1953	1956	Mean	1954	1955	Mean
80°F. continuous	86	78	82.0	65	65	65.0
95°F. continuous	90	75	82.5	60	37	48.5

The analysis of variance on percentage data transformed by means of the angular transformation (2) is given in Table 2. The high significance for treatments was due mainly to the 110°F. drying temperature, which always resulted in a germination below the 65 per cent minimum. Although there was a trend towards improved germination as the initial period at 80°F. was increased, it is evident that in all years any treatment involving the 110°F. temperature was too severe. Regression on the various periods at 80°F. with 95°F. follow-up until seed was dry showed a definite tendency for germination to improve with an increase in the length of time at the lower temperature. This tendency and the lower germinations from treatments involving 110°F. are illustrated in Figure 1.

Temperature differences between the 1953 and 1956 summers on the one hand, and 1954 and 1955 on the other, were marked, the former two years being much warmer. Examination of the heat unit data revealed an accumulation from April 1 to September 30 of 2,381 and 2,458 day degrees (base 42°F.) for the former 2 years, and 2,110 and 2,001 for the latter. The average accumulation of these two pairs of years is shown in Figure 2.

When the germination data were grouped according to these seasonal differences in temperature, marked tendencies were observed. The difference between the 1953, 1956 pair and the 1954, 1955 pair, however, was not quite significant (Table 2), possibly due to the few degrees of freedom for the appropriate error. Table 3 shows the germination at 80°F. and 95°F. continuous for each of these pairs of years, and illustrates a marked tendency for better germination at the higher temperature in the warmer seasons.

It appears evident that in the two warmer years, seasonal temperatures favourably influenced maturity of the seed crop and, under such conditions,

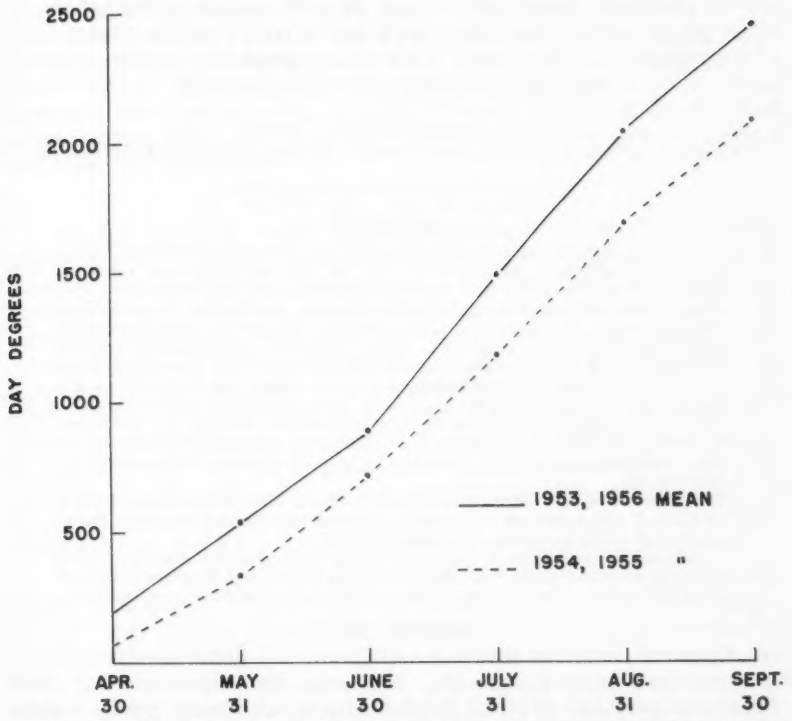

FIGURE 2. Day degrees (base 42°F.) accumulated at Saanichton, B.C., from April 1 to September 30, averaged for the years 1953 and 1956, and 1954 and 1955.

it is evident that higher drying temperatures can be safely employed. Based upon the results obtained in this study, a drying temperature of 95°F. is suggested where temperature conditions such as occurred in 1953 and 1956 prevail, and 80°F. continuous, if they are of the order of those which occurred in 1954 and 1955.

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Thanks are due also to D. R. Cook, Plotman, Experimental Farm, Saanichton, B.C., for technical assistance.

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COMMON ROOTROT AND PLANT DEVELOPMENT FOLLOWING TREATMENTS OF WHEAT SEED WITH ALDRIN, GAMMA BHC, AND HEPTACHLOR, WITH AND WITHOUT MERCURY FUNGICIDES[1]

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ABSTRACT

Common rootrot in Chinook wheat seedlings was more pronounced after seed treatments with gamma BHC at 1 oz. per bu. than after aldrin or heptachlor at the same rate or no insecticide, in soils inoculated with *Cochliobolus sativus* (Ito and Kurib.) Drechs. ex Dastur in greenhouse and field tests. In one of four tests rootrot was greater after heptachlor than after aldrin seed treatments. Disease increased progressively with an increase in rate of application of gamma BHC, but not of heptachlor, from ½ to 1 oz. per bu. Reduced emergence and seedling weight following the higher rates of gamma BHC apparently were caused by phytotoxicity of the seed dressings rather than by the increases in rootrot. In field tests rootrot frequently was greater, and emergence less, without insecticides than with most insecticides, possibly because of damage to the plants by wireworms.

The inclusion of a mercury fungicide with the insecticide, except gamma BHC at ¼ and 1 oz. per bu., usually reduced rootrot and increased emergence and seedling weight in inoculated soils.

Wheat plants apparently recovered from early stunting associated with phytotoxicity and rootrot as there were no differences in grain yield per plant between seed treatments or between inoculated and uninoculated soils.

INTRODUCTION

Common rootrot is the most widespread and destructive root disease of cereals in Western Canada (2). It is caused by certain fungi, of which *Cochliobolus sativus* (Ito and Kurib.) Drechs. ex Dastur, whose conidial stage is known as *Helminthosporium sativum* P.K. and B. (*Bipolaris sorokiniana* (Sacc. in Sorok.) Shoemaker), is considered the most important.

Some beneficial effects of organic mercury seed treatments on rootrot in cereals have been reported in the Prairie Provinces (2, 3, 4, 7) and in Holland (1), though they are not recommended for the control of rootrot in Saskatchewan. The effects of insecticides on common rootrot have received relatively little attention. Wallace[3] noted in one test, but not in another, that a gamma BHC (gamma isomer of 1,2,3,4,5,6-hexachlorocyclohexane) seed treatment encouraged rootrot infection in wheat seedlings. Richardson (5) found that soil treatments with aldrin (1,2,3,4,10,10-hexachloro-1,4,4a,5,8,8a-hexahydro-1,4,5,8-dimethanonapthalene), gamma BHC, and heptachlor (1,4,5,6,7,8,8-heptachlor-3a,4,7,7a-tetrahydro-4,7-methanoindene) were different in their effects on plant development and rootrot infection in barley seedlings. He observed also that none of these insecticides was toxic to *C. sativus* in culture.

In the Prairie Provinces seed treatments with aldrin, gamma BHC, or heptachlor, are widely used on cereal seed for the control of wireworms. They are often combined with mercury fungicides for the control of certain

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diseases as well. At Saskatoon in 1958, in greenhouse wireworm control tests, common rootrot was generally more pronounced in wheat seedlings grown from seed treated with gamma BHC than in those grown from seed treated with aldrin or heptachlor or no insecticide. Consequently, greenhouse and field tests were conducted in 1959 to learn more of the effects of aldrin, gamma BHC, and heptachlor seed treatments on common rootrot and wheat plant development.

MATERIALS AND METHODS

All seed dressings were commercial wettable powder formulations applied dry to registered Chinook wheat. Rates of application of insecticides are given in Tables 1 to 6 inclusive. In all tests each insecticide was applied with and without a mercury fungicide. Insecticide-only dressings contained 50 per cent* of the toxicant. Insecticide-fungicide dressings contained 40 per cent of the insecticide toxicant and 2 per cent mercury equivalent (Phenyl mercury acetate and ethyl mercury chloride), except gamma BHC which contained 30 per cent of the gamma isomer of BHC. With 1, 1, and ¾ ounces per bushel of aldrin, heptachlor, and gamma BHC respectively, the rate of application of mercury equivalent was 1/20 ounce per bushel; this varied as the rate of insecticide was increased or decreased. Mercury-only dressings contained 5 per cent mercury equivalent and were applied at 1/20 ounce of mercury equivalent per bushel.

Test 1 Greenhouse (Table 1) was conducted in light loam soil naturally infested with about 15 viable spores of *C. sativus* per gram**. No fungus inoculum was added. Tests 2 and 3 Greenhouse (Tables 2, 3) were in silty loam soil naturally infested with about five spores of *C. sativus* per gram. The soil was inoculated with water suspensions of spores of each of three laboratory cultured monoascosporic strains of *C. sativus* as follows: Test 2, approximately 1,000 spores of Isolate 73 and of Isolate T4 per gram of soil in each case; Test 3, 250 spores of Isolate 73 and 200 spores of Isolate TP13 per gram in each case. Each spore suspension was mixed thoroughly with the soil, and the soil moisture content was brought to 17 per cent on an oven-dry basis. Each treatment was replicated six, three, and four times in Tests 1, 2, and 3 respectively. A treatment replicate consisted of 25 seeds planted 2 inches deep in soil in a 7-inch clay pot. Where inoculated soil was used it extended from at least ½-inch below seed level to the surface.

In each field test (Tables 4, 5, 6) seed treated with each dressing was planted with and without an oat-hull inoculum of a composite of highly virulent strains of *C. sativus*. The inoculum was planted with the grain from a V-belt rod-row seeder. Each treatment was replicated four times in randomized blocks in each test. A treatment plot consisted of three rows, each 20 feet long and 9 inches apart, with 300 seeds in the centre row and 150 in each of the two outside rows. Tests 4 and 6 (Tables 4, 6) were conducted in silty clay loam soil at Saskatoon, and Test 5 (Table 5), a duplicate of Test 4, in light loam soil at Swift Current.

*Formulation percentages given are by weight.

**The authors are indebted to S. Chinn, Plant Pathology Section, Canada Department of Agriculture Research Station, Saskatoon, Sask., for estimating counts of *C. sativus* spores in soil samples.

TABLE 1. — NUMBERS AND COMMON ROOTROT DISEASE RATINGS OF CHINOOK WHEAT SEEDLINGS GROWN FROM SEED TREATED WITH ALDRIN, GAMMA BHC, AND HEPTACHLOR, EACH WITH AND WITHOUT A MERCURY FUNGICIDE, IN SOIL NATURALLY INFESTED WITH *C. sativus*. TEST 1, GREENHOUSE, SASKATOON, SASK., 1959

Treatment	Emerged plants	Disease rating
Insecticides		
Aldrin 1 oz./bu.	21.67	68.67
Gamma BHC 1 oz./bu.	15.00	79.92
Heptachlor 1 oz./bu.	20.00	63.42
No insecticide	21.25	63.75
L.S.D. (P = .05)	3.09	8.36
Fungicides		
With mercury fungicide	19.88	70.46
Without mercury fungicide	19.08	67.42
L.S.D. (P = .05)	—	—

TABLE 2. — NUMBERS, COMMON ROOTROT DISEASE RATINGS, AND DRY WEIGHTS OF CHINOOK WHEAT SEEDLINGS GROWN FROM SEED TREATED WITH ALDRIN, GAMMA BHC, AND HEPTACHLOR, EACH WITH AND WITHOUT A MERCURY FUNGICIDE, IN SOIL INOCULATED WITH TWO STRAINS OF *C. sativus* (73, WEAKLY VIRULENT; T4, HIGHLY VIRULENT) AND IN UNINOCULATED SOIL. TEST 2, GREENHOUSE, SASKATOON, SASK., 1959

Treatment	Emerged plants	Disease rating	Wt. per plant mgm.
Insecticides			
Aldrin 1 oz./bu.	21.78	42.22	219.5
Gamma BHC 1 oz./bu.	15.06	63.00	212.8
Heptachlor 1 oz./bu.	21.11	47.67	225.0
No insecticide	21.50	43.39	224.8
L.S.D. (P = .05)	1.09	5.30	—
Fungicides			
With mercury fungicide	20.19	46.73	222.9
Without mercury fungicide	19.55	51.41	218.1
L.S.D. (P = .05)	—	3.75	—
Inocula of *C. sativus*			
Inoculum 73	20.67	45.94	224.0
Inoculum T4	18.37	88.46	215.6
No inoculum	20.54	12.79	222.1
L.S.D. (P = .05)	0.95	4.59	—

In greenhouse tests, plant emergence, disease ratings and dry weights were determined from all emerged plants about 3 weeks after seeding. Disease ratings were based on lesions of the coleoptiles and basal parts of leaves: each plant was rated (numerical value in brackets) clean (0 – no lesions), slight (2 – few small lesions), moderate (5 – extensive lesions), or severe (10 – very extensive lesions or a lesion girdling the coleoptile or basal parts of the leaves), and the mean rating for a group determined as $\frac{\text{the total of all ratings}}{\text{number of plants}} \times 10$. Dry weights were obtained by severing plant tops from the roots and seed, drying them for 24 hours at 80°C., and weighing them.

TABLE 3. — NUMBERS, COMMON ROOTROT DISEASE RATINGS, AND DRY WEIGHTS OF CHINOOK WHEAT SEEDLINGS GROWN FROM SEED TREATED WITH VARIOUS RATES OF GAMMA BHC EACH WITH AND WITHOUT A MERCURY FUNGICIDE, IN SOIL INOCULATED WITH TWO STRAINS OF *C. sativus* (73, WEAKLY VIRULENT; TP13, MODERATELY VIRULENT) AND IN UNINOCULATED SOIL. TEST 3, GREENHOUSE, SASKATOON, SASK., 1959

Treatment	Emerged plants	Disease rating	Wt. per plant mgm.
Insecticides			
Gamma BHC 0 oz./bu.	21.25	28.20	240.1
½ oz./bu.	20.92	30.62	245.0
¾ oz./bu.	18.79	35.25	232.9
1 oz./bu.	14.96	37.12	220.0
L.S.D. (P = .05)	1.26	5.14	10.5
Fungicides			
With mercury fungicide	19.06	28.54	239.7
Without mercury fungicide	19.10	37.06	229.5
L.S.D. (P = .05)	—	3.65	7.4
Inocula of *C. sativus*			
Inoculum 73	19.31	33.41	241.8
Inoculum TP13	18.81	54.59	221.0
No inoculum	19.12	10.41	241.0
L.S.D. (P = .05)	—	4.45	9.1

TABLE 4. — NUMBERS, COMMON ROOTROT DISEASE RATINGS AND DRY WEIGHTS PER PLANT OF SEEDLINGS, AND GRAIN YIELD PER PLANT, OF CHINOOK WHEAT GROWN FROM SEED TREATED WITH ALDRIN, GAMMA BHC AND HEPTACHLOR EACH WITH AND WITHOUT A MERCURY FUNGICIDE, WITH AND WITHOUT AN OAT-HULL INOCULUM OF HIGHLY VIRULENT STRAINS OF *C. sativus*. TEST 4, FIELD, SASKATOON, SASK., 1959

Treatment	Emerged plants	Disease rating	Wt. per plant mgm.	Yield per plant gm.
Insecticides				
Aldrin 1 oz./bu.	221.1	27.99	208.9	2.85
Gamma BHC 1 oz./bu.	206.2	35.40	158.9	2.95
Heptachlor 1 oz./bu.	211.5	28.89	201.8	2.86
No insecticide	195.3	27.83	206.0	2.96
L.S.D. (P = .05)	10.8	4.76	28.0	—
Fungicides				
With mercury fungicide	227.7	27.09	186.4	2.81
Without mercury fungicide	189.4	32.95	201.3	3.00
L.S.D. (P = .05)	7.6	3.36	—	—
Inocula of *C. sativus*				
With inoculum	203.5	34.20	179.6	2.90
Without inoculum	213.6	25.85	208.4	2.91
L.S.D. (P = .05)	7.6	3.36	17.6	—

In field tests emergence counts of plants were made from the two outside rows of each plot, at least 1 month after seeding. Disease ratings were determined from 50 plants taken randomly, and dry weights of plants were determined from all the plants from one outside row 1 month after seeding.

Grain yields were obtained from field plots, except those at Swift Current (Table 5), by harvesting and threshing all heads in the centre row

of each plot. In Test 4 (Table 4) the centre rows were thinned in June to about 100 plants per row, and the plants were counted after harvest to give the grain yield per plant. In Test 6 (Table 6) the plants were not thinned, or counted after harvest, and only the total yields per row were obtained.

Factorial analyses of the data were conducted to detect differences in plant emergence, disease rating, seedling dry weight, and grain yield between insecticides, between fungicide and no fungicide, and between the different inocula of *C. sativus*. Analyses of covariance were applied where necessary to detect and remove the effects of plant numbers on seedling weight and on grain yield.

RESULTS

The means for each of the main effects (insecticides, fungicides, and inocula of *C. sativus*), averaged over both other main effects, are given in Tables 1 to 6 inclusive. For example, the number of emerged plants for aldrin (21.78) in Table 2 is the average number of emerged plants per plot for all plots in which aldrin seed treatments occur (with and without a mercury fungicide and with and without inocula of *C. sativus*). Interactions between insecticides, fungicides, and inocula are discussed as qualifications of the main effects.

Insecticides

In all tests except Test 5 Field (Table 5), common rootrot was more pronounced in seedlings from seed treated with gamma BHC at 1 ounce per bushel than in seedlings from seed treated with aldrin or heptachlor at the same rate or no insecticide (Tables 1, 2, 3, 4, 6). Rootrot increased progressively with an increase in rate of gamma BHC, but not of heptachlor, from ½ to 1 ounce per bushel (Tables 3, 6). The differences in rootrot

TABLE 5. — NUMBERS, COMMON ROOTROT DISEASE RATINGS AND DRY WEIGHTS PER PLANT OF CHINOOK WHEAT SEEDLINGS GROWN FROM SEED TREATED WITH ALDRIN, GAMMA BHC AND HEPTACHLOR EACH WITH AND WITHOUT A MERCURY FUNGICIDE, WITH AND WITHOUT AN OAT-HULL INOCULUM OF HIGHLY VIRULENT STRAINS OF *C. sativus*. TEST 5, FIELD, SWIFT CURRENT, SASK., 1959

Treatment	Emerged plants	Disease rating	Wt. per plant mgm.
Insecticides			
Aldrin 1 oz./bu.	219.8	35.19	155.7
Gamma BHC 1 oz./bu.	193.9	40.75	136.9
Heptachlor 1 oz./bu.	217.9	36.00	167.7
No insecticide	203.3	38.37	156.9
L.S.D. (P = .05)	13.0	5.20	19.2
Fungicides			
With mercury fungicide	219.2	32.34	160.0
Without mercury fungicide	198.2	42.81	148.6
L.S.D. (P = .05)	9.2	3.67	—
Inocula of *C. sativus*			
With inoculum	192.5	43.78	149.0
Without inoculum	225.0	32.50	159.6
L.S.D. (P = .05,	9.2	3.67	—

ratings between gamma BHC and the other insecticides generally were significant both when the insecticides were applied alone and when they were combined with the mercury fungicides, but they were greater in the latter case.

Emergence and seedling dry weights decreased progressively with an increase in rate of application of gamma BHC, but not of heptachlor. Interactions between insecticides and fungicides showed, however, that these adverse effects of gamma BHC followed mainly the BHC-fungicides treatments rather than gamma BHC alone.

Emergence, disease ratings, and seedling dry weights were similar following aldrin and heptachlor seed treatments except that disease ratings were higher in Test 2 (Table 2) following heptachlor than following aldrin. In field tests emergence was lower and/or disease ratings were higher after no insecticide than after some insecticide treatments (Tables 4, 5, 6). The results from aldrin in all categories were as good as or better than those from gamma BHC, heptachlor, or no insecticide.

There were no differences in the grain yields per plant between the different seed treatments (Table 4). The average grain yield per row for all rates of heptachlor was higher than that for the same rates of gamma BHC (Table 6).

Differences in emergence, seedling dry weights, and grain yields between insecticide seed treatments were no greater in inoculated soil than in uninoculated soil, indicating that these differences were caused by the phytotoxicity of the seed treatments and not by differences in rootrot infection.

TABLE 6. — NUMBERS, COMMON ROOTROT DISEASE RATINGS AND DRY WEIGHTS OF SEEDLINGS, AND TOTAL GRAIN YIELDS, OF CHINOOK WHEAT GROWN FROM SEED TREATED WITH GAMMA BHC AND HEPTACHLOR EACH WITH AND WITHOUT A MERCURY FUNGICIDE, WITH AND WITHOUT AN OAT-HULL INOCULUM OF HIGHLY VIRULENT STRAINS OF *C. sativus*. TEST 6, FIELD, SASKATOON, SASK., 1959

Treatment	Emerged plants	Disease rating	Wt. per plant mgm.	Total grain yield gm.
Insecticides				
Gamma BHC ½ oz./bu.	217.1	29.19	190.5	318.3
¾ oz./bu.	205.4	35.69	161.5	305.5
1 oz./bu.	197.9	41.12	153.9	292.9
Heptachlor ½ oz./bu.	212.5	31.94	181.4	350.4
¾ oz./bu.	200.3	31.62	191.8	338.2
1 oz./bu.	205.7	31.94	200.4	315.6
No insecticide	203.7	37.12	176.8	310.9
L.S.D. (P = .05)	11.8	4.74	32.3	—
Fungicides				
With fungicide	218.8	30.91	173.1	320.1
Without fungicide	193.4	37.27	182.8	317.6
L.S.D. (P = .05)	6.3	2.43	—	—
Inocula of *C. sativus*				
With inoculum	201.3	42.57	163.3	301.6
Without inoculum	210.9	25.61	195.7	336.1
L.S.D. (P = .05)	6.3	2.43	17.3	23.1

Fungicides

Rootrot ratings were lower following insecticide-fungicide seed treatments than following insecticides alone in two greenhouse tests (Tables 2, 3) and in all field tests (Tables 4, 5, 6). Insecticide-fungicide and fungicide-inocula interactions showed that the fungicides generally were beneficial with all insecticides, except gamma BHC at ¾ and 1 ounce per bushel, in inoculated soils. An increase in emergence usually accompanied a reduction in rootrot. In one greenhouse test (Table 3), and in one field test in inoculated soil (Table 5), seedling dry weights were higher after treatments that included fungicides than after those that did not. The presence or absence of the fungicides with each of the insecticides in the seed treatments did not increase or decrease grain yield.

Inocula of C. sativus

Emergence and seedling dry weights generally were lower, and disease ratings higher, in inoculated soils than in uninoculated soils. In greenhouse tests these differences were apparent with and without mercury fungicides in the seed treatments, but in field tests they occurred mainly where no fungicides were included. There was no difference in grain yield per plant between inoculated and uninoculated soil (Table 4), but the grain yield per row was higher in uninoculated soil (Table 6) probably because of the greater number of emerged plants.

DISCUSSION

The results indicate that the phytotoxicity of the gamma BHC seed treatments predisposed the wheat plants to common rootrot infection. Also, the accompanying reductions in emergence and seedling weights apparently resulted from phytotoxicity rather than from the increases in rootrot infection. A comparison of the results from inoculated and uninoculated soils provides ample evidence that rootrot infection can reduce emergence and seedling weight. However, the increase in disease brought about by gamma BHC either had no effects or the effects were masked by symptoms of phytotoxicity. Heptachlor treatments occasionally resulted in similar, though very slight, effects. None of the aldrin treatments increased disease ratings or decreased plant emergence or seedling dry weights.

The occasionally lower emergence or higher rootrot infection following no insecticides in seed treatments in field tests, compared to those following insecticides, are not readily explainable. There was occasional wireworm damage in the plots, and possibly mechanical damage by wireworms or other organisms killed some plants directly and provided easy access for the rootrot fungi in others.

The data do not indicate any stimulation of seedling growth by gamma BHC and heptachlor, increase in the severity of rootrot infection by heptachlor, or reduction of rootrot infection by aldrin, as reported by Richardson (5) with barley seedlings in soil treated with these insecticides. However, Richardson pointed out that his results could not be explained in all cases by a direct action of the chemicals on the causal organism, and that possibly the chemicals alter the metabolism of the host in a way which increases or decreases its resistance to the disease. From this point

of view, differences in formulation, amount, and distribution of the insecticides, in soil characteristics, or in the host plant, between the soil and seed treatments, could be responsible for the differences in results.

Apparently the plants recovered from early stunting brought about by phototoxicity of the chemicals or by rootrot infection, as yields of grain did not reflect reductions in seedling weights. Sallans (7) found that Reward wheat made notable recovery from early infections by *C. sativus*. General field observations have indicated that wheat plants frequently recover from early stunting caused by phototoxicity of seed treatments. By inference, however, the effects of the seed treatments and of disease on total yield were then the same as their effects on emergence. This inference is supported in part in the test where the harvested plants were not thinned in the seedling stage, where generally the grain yield increased with an increase in the number of emerged plants (Table 6).

The likelihood of severe rootrot infection, and of its adversely affecting plant development, probably was greater in the tests described than in natural field conditions. In the tests the fungus spores were distributed either around the seed or throughout the soil, allowing for early and continuous infection. In Saskatchewan natural infection in the field arises mainly from spores on or in the surface soil, where the seedlings are not infected until they have made some growth. The experimental results serve mainly to show that phytotoxicity of insecticide seed treatments may affect common rootrot infection. The significance of this effect under natural field conditions remains to be shown.

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INSECTICIDE RESIDUES IN POTATOES AFTER SOIL TREATMENTS FOR CONTROL OF WIREWORMS[1]

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ABSTRACT

Chemical analyses and insect bioassays showed residues of less than 0.1 p.p.m. of aldrin, chlordane, dieldrin, and heptachlor in the pulp of early potatoes grown in a Berrien sandy loam soil treated for control of the eastern field wireworm, *Limonius agonus* (Say). The insecticides had been applied at the rate of 5 pounds of toxicant per acre, 3, 15, or 27 months before harvesting the potatoes. Mammalian feeding trials showed that the residues in the potatoes did not cause any gross or histopathological changes in guinea pigs and rats.

INTRODUCTION

In 1951, 1952, and 1953, nine soil insecticides were applied for control of the eastern field wireworm, *Limonius agonus* (Say), attacking early potatoes in a field near Chatham, Ontario. This is a report on mammalian feeding trials, insect bioassays, and chemical analyses for residues of aldrin[5], chlordane[6], dieldrin[7], and heptachlor[8] in or on the potatoes grown in the treated soil. As reported elsewhere (2), only these four materials of those tested at that time gave satisfactory control of wireworms attacking potatoes in southwestern Ontario. The other materials tested, including DDT[9], parathion[10] and BHC containing 12[11], 90[11], and 100[12] per cent *gamma* isomer, either did not control the wireworms satisfactorily at the rates applied or produced off-flavour in the tubers.

MATERIALS AND METHODS

The experiment was conducted in a field of Berrien sandy loam severely infested with the eastern field wireworm, *L. agonus*. Potatoes grown in this field in 1949 and 1950 were unmarketable because of wireworm injury.

Three adjacent ranges, each of four randomized blocks of plots, 20 feet x 20 feet, were established in the field. One range was treated in 1951, another in 1952, and the third in 1953. The materials (Table 1) were applied as suspensions at 5 pounds of toxicant in 108 gallons of water per acre. The dates of application were: June 2, 1951; May 16, 1952; and

[1]Contribution from the Entomology Division (No. 3888) Science Service, and from the Animal Pathology Division, Production Service, Canada Department of Agriculture, Ottawa, Ont.; from a thesis submitted by the senior author in partial fulfilment of requirements for a M.Sc. degree at the University of Western Ontario.

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[5]1,2,3,4,10,10-hexachloro-1,4,4a,5,8,8a-hexahydro-1,4-*endo*, *exo*-5,8-dimethanonaphthalene, 20% wettable powder. (Julius Hyman Co., Denver, Colo.).

[6]1,2,4,5,6,7,8,8-octachloro-3a,4,7,7a-tetrahydro-4,7-methanoindan, 40% wettable powder. (Dow Chemical Co., Midland, Mich.).

[7]1,2,3,4,10,10 - hexachloro-*exo*-6, 7-epoxy-1,4,4a,5,6,7,8,8a-octahydro-1,4-*endo*,*exo*-5,8-dimethanonaphthalene, 25% wettable powder. (Julius Hyman Co., Denver, Colo.).

[8]1 (or 3a), 4,5,6,7,8,8-heptachloro-3a,4,7,7a-tetrahydro-4, 7-methanoindene, 25% wettable powder. (Velsicol Corp., Chicago, Ill.).

[9]A complex mixture, mainly of 1,1,1-trichloro-2,2-bis (*p*-chlorophenyl) ethane, 50% wettable powder. (Canadian Industries Ltd., Montreal, Que.).

[10]0,0-diethyl 0-*p*-nitrophenyl phosphorothionate, 15% wettable powder. (Cyanamid of Canada Ltd., Toronto, Ont.).

[11]Mixed isomers of 1,2,3,4,5,6-hexachloro*cyclo*hexane, 50% wettable powder. (Canadian Industries Ltd., Montreal, Que.).

[12]*Gamma* isomer of 1,2,3,4,5,6-hexachloro*cyclo*hexane (minimum purity 99%), 25% wettable powder. (Dow Chemical Co., Midland, Mich.).

May 26, 1953. The treatments were double-disked into the soil as soon as possible after application.

Irish Cobbler potatoes were planted in the treated plots with a mechanical planter on the following dates: June 8, 1951; May 21, 1952; and May 27 and 28, 1953.

The tubers from 14 feet of each of the four centre rows of each plot were harvested by hand approximately 12 weeks after planting. The Canada No. 1 grade-size potatoes were stored in new jute bags, each treatment separately, to minimize contamination.

Mammalian feeding trials, insect bioassays, and chemical determinations for insecticide residues in or on representative samples of the potatoes grown in the treated plots were conducted with the assistance of the following institutions:

(a) *Animal Diseases Research Institute, Canada Department of Agriculture, Hull, Quebec*

The following portions of potatoes grown in 1951 in the plots treated with the four insecticides were each fed to 6 male guinea-pigs: washings from the daily ration of potatoes, the corresponding peeling, the outer layer of peeled potato, and the central parts of tuber tissue. Thus, a total of 24 male guinea-pigs were used for each of the four treated groups of potatoes. The control group, consisting of 18 male guinea-pigs, was divided into three groups of 6 animals each, and received corresponding portions of the untreated potatoes, excepting that the tuber tissue was not separated into outer layer and central part for the test-feeding.

The potatoes treated in 1953, as well as the untreated potatoes of that year, were each fed to 24 female rats.

All experimental animals were mature; the male guinea-pigs varied in weight from 450 to 520 grams. They were randomly selected from the large colony at the Animal Diseases Research Institute. The white female rats were of the Sprague-Dawley strain, bred for insecticidal studies and supplied for these feeding trials by the Occupational Health Laboratory, Department of National Health and Welfare. The initial weights varied from 240 to 280 grams. In subdividing these randomly selected animals into groups of 6, undue deviations of individual weights from the mean were avoided. By doing this, the calculated average daily intake per animal was expected to be a close approximation of the actual intake of each individual animal.

In 1951, the daily rations for the guinea-pigs consisted of 30 grams of potato-peeling, tuber tissue, or washing. The tuber washings for the groups of 6 animals were prepared by carefully washing all potatoes needed for the day's feeding with 180 ml. distilled water. About 2 hours after the four portions of the potatoes were offered, the regular diets were given *ad libitum*. On Sundays and holidays the guinea-pigs received their normal diets.

Potatoes treated as indicated were fed to the test animals as follows: aldrin, 78 feedings within a period of 94 days; chlordane, 56 feedings within 66 days; dieldrin, 66 feedings within 80 days; and heptachlor 76

feedings within 92 days. The untreated potatoes were fed 81 times during a period of 98 days. The duration of the trials varied because of spoilage of some tubers due to slight freezing in sub-zero weather during transit and storage.

In 1953, the rats were given similar samples of the potatoes in 20-gram amounts and those fed with the various portions of the tubers received no other food during daylight; moderate amounts of water were offered to those receiving the fairly dry potato peelings. At night, the standard ration was supplied and water fountains were replenished. Groups that received the potato washings were fed the otherwise normal diet and fresh water was given after the washings were consumed. Normal diets were fed on Saturdays, Sundays and holidays. No spoilage of potatoes had occurred, and 81 to 86 feedings were made during a period of 115 to 123 days.

The average intake per animal in each group was calculated daily by re-weighing the potato portions remaining from the previous day's feeding and allowing for a loss of approximately 20 per cent in weight for dehydration.

The test animals were observed closely for symptoms of insecticidal poisoning, and were weighed bi-weekly.

Autopsies were performed on animals that died during the experiments. Specimens of the principal organs were preserved for histological examination. At the conclusion of the trials, all animals were bled under ether anaesthesia for autopsy. In 1951, at least 3 guinea-pigs of each group were reserved for special histological examination. During autopsy any gross pathological changes were noted, and samples of 13 principal organs were removed for histological examination. These tissues, which included the lungs, heart, liver, spleen, kidneys, thyroid, adrenals, pancreas, stomach, small intestines, caecum, urinary bladder, and cerebrum, were fixed in 10 per cent formal-saline. The sections, stained with haematoxylin-eosin, were studied without references to gross pathology, if present.

(b) *Chemistry Section, Science Service Laboratory, Canada Department of Agriculture, Kentville, Nova Scotia*

In 1951 and 1952, various portions of potatoes were chemically analysed for aldrin (4) and chlordane (5). Analyses were also made of the soil that adhered to the tubers when harvested. In 1952, an oily residue interfered with the analyses, making these results unreliable.

(c) *Velsicol Corporation, Chicago, Illinois*

In 1951, 1952, and 1953, analyses were conducted for chlordane residues by the modified Davidow spectrophotometric method (5). The modified Polen-Silverman method (14) was used in analysing for heptachlor.

(d) *Julius Hyman and Company, or Julius Hyman and Company Division, Shell Chemical Corporation, Denver, Colorado*

In 1951 and 1952, insect bioassays were conducted for aldrin and dieldrin residues. The method used was adapted from that described by Sun and Sun (16). This method was sensitive to 0.1 p.p.m. of toxicant.

TABLE 1.—RESIDUES (P.P.M.)[1] FOUND BY CHEMICAL ANALYSES IN OR ON POTATOES GROWN IN SOIL TREATED WITH 5 POUNDS OF TOXICANT PER ACRE OF ONE OF FOUR INSECTICIDES, CHATHAM, ONTARIO

Material tested	Year of application	Year potatoes grown	Aldrin	Chlordane	Dieldrin[2]	Heptachlor[3]
Peel	1951	1951	Nil[4]	<1.0[4]	—	0.04
	1951	1952	<0.1[2]	—	—	—
	1951	1953	—	Nil[3]	—	Nil
	1952	1952	<0.1[2]	—	—	—
	1952	1953	—	0.01[3]	—	Nil
	1953	1953	—	0.08[3]	—	0.08
Pulp	1951	1951	Nil[4]	Nil[4]	—	Nil
	1951	1952	Nil[4]	—	—	—
	1951	1953	—	Nil[3]	—	Nil
	1952	1952	Nil[4]	—	—	—
	1952	1953	—	Nil[3]	—	Nil
	1953	1953	—	0.01[3]	—	0.01
Whole potato	1951	1951	—	0.01[3]	—	—
	1951	1952	—	0.01[3]	—	Nil
	1951	1953	<0.1[2]	0.04[3]	<0.1	0.01
	1952	1952	—	0.04[3]	—	Nil
	1952	1953	<0.1[2]	0.01[3]	<0.1	0.02
	1953	1953	<0.1[2]	0.03[3]	<0.1	0.07
Soil	1951	1951	Nil[4]	<0.5[4]	—	—

[1]Maximum residue reported when more than one sample analysed
[2]Julius Hyman and Company, or Julius Hyman and Company Division, Shell Chemical Corporation, Denver, Colo.
[3]Velsicol Corporation, Chicago, Ill.
[4]Chemistry Section, Science Service Laboratory, Canada Department of Agriculture, Kentville, N.S., method sensitive only to 1.0 p.p.m. of chlordane

TABLE 2. — RESIDUES (P.P.M.)[1] FOUND BY BIOASSAY IN OR ON POTATOES GROWN IN SOIL TREATED WITH 5 POUNDS OF TOXICANT OF ONE OF THREE INSECTICIDES, CHATHAM, ONTARIO

Material tested	Year of application	Year potatoes grown	Aldrin	Dieldrin	Heptachlor[2]
Peel	1951	1951	<0.1[2]	<0.1[2]	—
	1951	1952	—	<0.1[2]	—
	1951	1952	Nil[3]	0.04[3]	0.05
	1951	1953	Nil[3]	Nil[3]	Nil
	1952	1952	—	<0.1[3]	—
	1952	1952	0.07[3]	Nil[3]	0.06
	1952	1953	Nil[3]	0.09[3]	Nil
	1953	1953	Nil[3]	Nil[3]	Nil
Pulp	1951	1952	Nil[3]	Nil[3]	0.02
	1951	1953	Nil[3]	Nil[3]	—
	1952	1952	Nil[3]	0.02[3]	Nil
	1952	1953	Nil[3]	Nil[3]	Nil
	1953	1953	Nil[3]	Nil[3]	Nil
Whole potato	1951	1951	<0.1[2]	<0.1[2]	—
	1951	1952	<0.1[2]	<0.1[2]	—
	1952	1952	<0.1[2]	<0.1[2]	—
Potato washings	1951	1951	<0.1[2]	<0.1[2]	—
	1951	1952	—	0.3[2]	—
	1952	1953	—	<0.1[2]	—

[1]Maximum residue reported when more than one sample analysed
[2]Julius Hyman and Company, or Julius Hyman and Company Division, Shell Chemical Corporation, Denver, Colo.
[3]Entomology Laboratory, Chatham, Ont.

In 1952 and 1953, chemical analyses were made for aldrin residues by the phenyl-azide photometric method (4). In 1953, the reduction phenyl-azide photometric method (13) was used in analysing for dieldrin residues. These methods were sensitive to 0.1 p.p.m. of toxicant.

(e) *Entomology Laboratory, Science Service, Canada Department of Agriculture, Chatham, Ontario*

In 1952 and 1953, biological determinations were made for aldrin, dieldrin, and heptachlor residues with the pomace fly, *Drosophila melanogaster* Meig., as the test insect (7). The results were analysed statistically by the method of Litchfield and Wilcoxon (12).

RESULTS AND DISCUSSION

The insecticide residues in the peel and in the pulp of potatoes grown in soil treated with aldrin, chlordane, dieldrin, or heptachlor each appeared to be less than 0.1 p.p.m. of toxicant (Tables 1 and 2). Residues, when detected, were lower in the potato pulp than in the peel. The highest residues were found in the potato washings and in soil adhering to the tubers when harvested. Thus, the residues, when present, decreased in amount from outside to inside the tubers. Since potatoes are usually washed and peeled in preparation for cooking, surface contamination would be largely removed, leaving only pulp containing considerably less than 0.1 p.p.m. of insecticide.

The chemical analyses used for aldrin and heptachlor may not have detected all the insecticide residues. Gannon and Biggar (8) have found that aldrin is epoxidized in the soil to dieldrin and heptachlor to heptachlor epoxide. A similar conversion of heptachlor occurs on plant material but light appears to be necessary for the conversion (9). The information on epoxides was not available at the time these experiments were conducted.

In the feeding trials with tubers grown in 1951, none of the guinea-pigs exhibited symptoms attributable to the toxic action of aldrin, chlordane, dieldrin, or heptachlor. The rate of intake of the various portions of the treated and control potatoes was almost identical and the percentage increase in weight in the four test groups was, in general, similar to that of the controls. Only the guinea-pigs consuming peelings of potatoes grown in chlordane-treated soil showed increments below the average level (Table 3).

Some mortality was experienced in all four groups of test animals, as well as in the controls. The distribution of the fatalities showed no definite pattern; animals consuming any of the four portions of the various tubers succumbed in closely comparable numbers as indicated in Table 4. The total number of deaths amounted to 15 guinea-pigs: 1 animal of the aldrin group (4.2 per cent); 3 guinea-pigs, each, in the dieldrin and heptachlor group (12.5 per cent); and 4 guinea-pigs in the chlordane and control group (16.6 and 22.2 per cent, respectively). The cause of these deaths was presumed to be due to the feeding in the first week of the trial of potatoes which were slightly frozen during transit and storage. This factor became apparent only after several days of feeding, and death due to enteritis or gastroenteritis was disclosed in animals that died as early as 2 weeks

TABLE 3. — AVERAGE INTAKE AND GAIN IN WEIGHT OF GUINEA-PIGS FED VARIOUS PORTIONS OF POTATOES GROWN IN SOIL TREATED WITH 5 POUNDS OF TOXICANT OF ONE OF FOUR INSECTICIDES, CHATHAM, ONTARIO

Experimental group	Average intake animal/day		Average weights, grams		Gains in weight	
			Initial	Final	Grams	Per cent
Aldrin	Potato washings	30 ml.	477	723	246	51.6
	Potato peelings	28.2 gm.	453	711	258	56.9
	Potato pulp (outer)	29.2 gm.	468	665	197	42.1
	Potato pulp (inner)	29.8 gm.	472	772	300	63.6
Chlordane	Potato washings	30 ml.	477	694	217	45.5
	Potato peelings	27.9 gm.	518	646	128	24.7
	Potato pulp (outer)	29.5 gm.	468	704	236	50.4
	Potato pulp (inner)	29.6 gm.	473	721	248	52.4
Dieldrin	Potato washings	30 ml.	478	679	201	42.1
	Potato peelings	29.8 gm.	463	743	280	60.5
	Potato pulp (outer)	29.7 gm.	470	684	214	45.5
	Potato pulp (inner)	29.8 gm.	473	709	236	49.9
Heptachlor	Potato washings	30 ml.	478	675	197	41.2
	Potato peelings	29.5 gm.	463	744	281	60.7
	Potato pulp (outer)	29.8 gm.	503	735	232	46.1
	Potato pulp (inner)	29.8 gm.	475	672	197	41.5
Control	Potato washings	30 ml.	480	660	180	37.5
	Potato peelings	28.3 gm.	468	719	251	53.6
	Potato pulp	28.2 gm.	476	753	277	58.2

TABLE 4. — COMPLICATING INFECTIONS IN GUINEA-PIGS FED VARIOUS PORTIONS OF POTATOES GROWN IN SOIL TREATED WITH 5 POUNDS OF TOXICANT OF ONE OF FOUR INSECTICIDES, CHATHAM, ONTARIO

Experimental group	Death in weeks (complications)[1]			
	Potato washings	Potato peelings	Potato pulp, outer layer	Potato pulp, central part
Aldrin			2 (E.)	
Chlordane	5½ (Pn.)	4 (Ge.)		3 (Ge.) 4 (E.)
Dieldrin			11 (Pn.)	2½ (E.) 10 (Pn.)
Heptachlor	7½ (Ge.) 12 (Pn.)		11½ (E.)	
Control	7 (Pn.) 13 (Ge.)	3 (E.) 5½ (Ge.)		

[1]E. = enteritis; Ge. = gastroenteritis; Pn. = pneumonia.

after the start of the experiment. Pneumonia, combined with exudative pleuritis and pericarditis, accounted for 5 of the 15 deaths. Apart from histopathological changes related to the complicating factors or to autolysis, all organs of these animals appeared normal.

No abnormalities were found in the guinea-pigs which were sacrificed at the conclusion of the feeding trials. The only detected change in the 13 principal organs which were examined histologically from at least 3 of the surviving animals in each group was glomerular tubular nephritis in one guinea-pig fed dieldrin-treated peelings. This was regarded as not being significant.

The rats fed the potatoes grown in 1953 showed no behaviour indicative of any toxic action of the insecticides. Consumption of the various portions of the potatoes from the treated soil was similar in amount to that eaten by the controls. A somewhat lower intake of the peelings was noted in all groups and a corresponding lower gain in weights occurred in the animals consuming the peelings of the potatoes grown in dieldrin- and heptachlor-treated soil. Similarly, the intake of washings from dieldrin-treated potatoes resulted in decreased weight increments of the rats.

The weight gains of the remaining principal animals were closely comparable to those of the controls (Table 5).

Of the 120 rats used in the experiment, 4 died before completion of the trial. Two of the fatalities, occurring in the aldrin group, were due to pneumonia; one rat of the heptachlor group succumbed early in the trial to an undetermined bacterial infection, and one control rat to complications resulting from a diaphragmatic hernia. The tissue specimens collected from these animals exhibited no lesions that could be related to a toxic action of the insecticides. Only minor post-mortem changes were noted in the control rat.

TABLE 5. — AVERAGE INTAKE AND GAIN IN WEIGHT OF RATS FED VARIOUS PORTIONS OF POTATOES GROWN IN SOIL TREATED WITH 5 POUNDS OF TOXICANT OF ONE OF FOUR INSECTICIDES, CHATHAM, ONTARIO

Experimental group	Average intake animal/day		Average weights, grams		Gains in weight	
			Initial	Final	Grams	Per cent
Aldrin	Potato washings	20 ml.	288	326	38	13.2
	Potato peelings	16.2 gm.	273	311	38	13.9
	Potato pulp (outer)	19.4 gm.	259	310	51	19.7
	Potato pulp (inner)	18.6 gm.	246	287	41	16.7
Chlordane	Potato washings	20 ml.	288	320	32	11.1
	Potato peelings	16.6 gm.	276	318	42	15.2
	Potato pulp (outer)	19.0 gm.	264	300	36	13.6
	Potato pulp (inner)	17.9 gm.	246	274	28	11.4
Dieldrin	Potato washings	20 ml.	288	305	17	5.9
	Potato peelings	14.3 gm.	276	303	27	9.8
	Potato pulp (outer)	18.9 gm.	262	303	41	15.6
	Potato pulp (inner)	16.0 gm.	242	279	37	15.3
Heptachlor	Potato washings	20 ml.	283	317	34	12.0
	Potato peelings	15.2 gm.	272	291	19	6.9
	Potato pulp (outer)	19.4 gm.	263	296	33	12.5
	Potato pulp (inner)	17.0 gm.	242	273	31	12.8
Control	Potato washings	20 ml.	285	318	33	11.6
	Potato peelings	15.4 gm.	269	308	39	14.5
	Potato pulp (outer)	19.1 gm.	264	304	40	15.2
	Potato pulp (inner)	19.2 gm.	239	277	38	15.9

Autopsy of the 116 survivors revealed no gross pathological changes indicative of insecticidal action. Necrotic foci were found scattered through the parenchyma of the livers and spleens of the 6 rats fed the peripheral layer of dieldrin-treated tubers. Histological study of these lesions revealed them to be composed of necrotic centres surrounded by a granulomatous type of tissue reaction. The ethiological factor was not disclosed, but the fact that the changes were confined to the organs of but one isolated group of rats was suggestive evidence of an infective agent.

All tissues of the remaining 110 rats were within the normal histological range.

Other investigators found that the toxic levels of these insecticides for rats were considerably higher than the residues reported in this paper. Dutra (6) reported that diets containing 5.0 p.p.m. of aldrin produced no lesions, whereas diets containing 75.0 p.p.m. produced changes in central zones of the liver. Diets containing more than 50.0 p.p.m. of dieldrin produced degenerative changes in liver, but changes were not observed with diets containing 25.0 p.p.m. (6). Ambrose *et al.* (1) found no cytological changes in liver of rats fed on diets containing chlordane below levels of 30.0 to 50.0 p.p.m. Rats survived for 6 months on a diet containing 30 p.p.m. of heptachlor, although this chemical is deposited in fat at dietary levels exceeding 0.1 p.p.m. (15).

The Food and Drug Directorate of the Canada Department of National Health and Welfare has established the following tolerances in potatoes: 0.1 p.p.m. for dieldrin and heptachlor and 0.3 p.p.m. for chlordane (17). A residue tolerance has not yet been established for aldrin on potatoes, although this material has been registered for use in Canada. In the United States, the following residues are allowed in potatoes: 0.1 p.p.m. for aldrin, 0.3 p.p.m. for chlordane (10, p. 1507), 0.1 p.p.m. for dieldrin (11, p. 2465) and zero for heptachlor (3, p. 613).

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EFFECTS OF CHEMICAL TREATMENT AND STORAGE ON SUNFLOWER SEEDS[1]

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ABSTRACT

Seeds of Sunrise sunflowers were treated with 14 common chemical seed dressings at 1½ and 15 ounces per bushel and stored up to 4½ years. At intervals samples of seeds were sown in soil beds in a greenhouse, and plated on agar in petri dishes.

Germinability of stored seed was not affected adversely by treatment with 1½ ounces per bushel of any chemical tested. Treatment with 15 ounces per bushel of some mercurial preparations markedly reduced germination, and induced characteristic symptoms of mercury poisoning in surviving seedlings. No injury was caused by the non-mercurial materials. Growth of fungi from treated seeds plated on agar was effectively prevented by most of the mercurial chemicals even at 1½ ounces per bushel. Several of the non-mercurial materials reduced the number of fungus colonies, but none gave complete control. The microflora appeared to be confined primarily to the hulls of sunflower seeds, although some invasion of the testa also occurred.

INTRODUCTION

Chemical treatment to control seed-borne disease is a well established practice in plant pathology. A great deal of experimental work has been done with the seeds of many crops to provide a sound basis for the practice (5, 15, 16). Such experimental information is limited for sunflower seed. In a handbook for growers of sunflowers in Manitoba, Putt (10) in 1949 commented about seed treatment: "No conclusive data on the value of seed treatment for sunflowers has been secured. Generally speaking, seed treatment is good cultural practice for any crop and sunflowers should be no exception to this rule". Weibel in Illinois made practically the same comment in 1951 (17).

Published recommendations for chemical treatment of sunflower seeds to control disease are few. Mujica (7) in Chile stressed that no common seed disinfectants could destroy sclerotia of *S. sclerotiorum*, although some organic mercury dusts gave control of external seed-borne mycelium. Naumova (8) and Chuvahin (2) recommend disinfection of sunflower seeds as one of several measures to control *S. sclerotiorum* in the U.S.S.R. Where the soil is heavily infested with the pathogen, seed treatment is thought to afford protection against infection of the growing plant, rather than to control seed-borne inoculum. A leaflet provided in Uruguay in 1956 by Imperial Chemicals Limited ("Duperial" of Uruguay) recommends "Fernasan", the thiram product sold in North America as "Arasan", for treating sunflower seed.

Since large-scale commercial production of sunflowers was started in Manitoba in 1943, the most destructive diseases of sunflowers have been rust (*Puccinia helianthi* Schw.), leaf mottle or Verticillium wilt (*Verti-*

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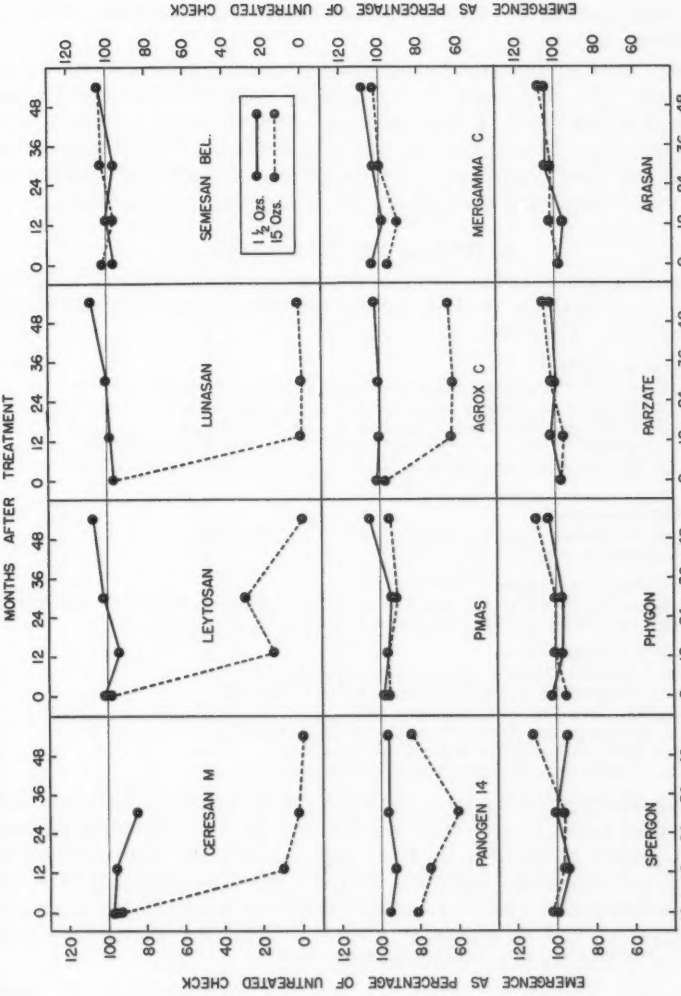

FIGURE 1. Effect of chemical seed dressing and subsequent storage on emergence of sunflowers.

cillium albo-atrum Reinke and Berth.), Sclerotinia wilt and root rot (*Sclerotinia sclerotiorum* (Lib.) de Bary), and downy mildew (*Plasmopara halstedii* (Farl.) Berl. and de Toni) (11, 12, 14). Contamination of sunflower seed by each of these organisms has been reported (3, 7, 9, 18), but positive transmission by seed has been shown experimentally only for *V. albo-atrum* (13).

When the work reported in this paper was begun in 1952, there was no evidence that any important pathogens were being disseminated on sunflower seed in Manitoba. It was, therefore, decided to try to obtain "conclusive data on the value of seed treatment for sunflowers" by testing various chemicals for their phytotoxicity, their effects on seed germination and longevity in storage, and their effects on the seed-borne microflora.

MATERIALS AND METHODS

The chemicals selected for the tests were representative of various types of seed dressings used in studies on cereal seeds and flax at this laboratory (5). They were as follows:

Organic Mercury Dusts:
Ceresan M, Ethyl mercury-toluene sulphonalide (3.2% mercury)
Half-ounce Leytosan, Phenyl mercury urea (4.5% mercury)
Lunasan, Ethyl mercury thiourea derivative (2.3% mercury)
Agrox C, Phenyl mercury acetate and mercury chloride (5% mercury)
Semesan Bel, Hydroxymercuronitrophenol 12% and hydroxymercurochlorophenol 2%

Organic Mercury Liquids:
Panogen 14, Methyl mercuridicyandiamide (1.4% mercury)
PMAS, Phenyl mercury acetate 10%

Insecticide Dusts plus Organic Mercury:
Mergamma C, (Lindane) Gamma isomer benzene hexachloride 40%, with phenyl mercury acetate and ethyl mercury chloride (2% mercury)

Non-mercurial Organic Dusts:
Arasan, (Thiram) Tetramethylthiuramdisulphide 50%
Spergon, (Chloranil) Tetrachloro-para-benzoquinone 98%
Phygon XL, Dichloronaphthaquinone 50%
Captan, N-trichloromethylthiotetrahydrophthalimide 75%
Thioneb 50W, Polyethylene thiuramsulphide 50%
Parzate, (Zineb) Zinc ethylene bisdithiocarbamate 65%.

The sunflower seed used was all of the variety Sunrise. The respective seed lots used were: 1) grown at Morden in 1951, and treated in January, 1952; 2) grown at Morden in 1951, and stored there until brought to Winnipeg and treated in December, 1952; 3) grown at Altona in 1952, and treated in December, 1952; 4) grown at Altona in 1953, and treated in December, 1953; and 5) grown at Altona in 1953, and stored there until brought to Winnipeg and treated in July, 1955.

The basic dosage rate used was the one recommended for many chemicals on flax, 1½ ounces per bushel. To determine possible phytotoxic effects of excessive dosage, each chemical was also applied at the rate of 15 ounces per bushel. The actual quantities of chemical were determined by the method described by Leukel in 1951 (4). The chemical was distributed uniformly on the seed by vigorous shaking. The treated seed was stored in tightly-stoppered glass containers.

TABLE 1. — EFFECT OF STORAGE ON EMERGENCE OF FIVE LOTS OF UNTREATED SUNFLOWER SEEDS

Lot 1		Lot 2		Lot 3		Lot 4		Lot 5	
Storage, months	Emergence, per cent	Storage, months	Emergence, per cent	Storage, months	Emergence, per cent	Storage, months	Emergence, per cent	Storage, months	Emergence, per cent
0	95	0	85	0	97	1	78	1	79
4	89	1	96	1	100	18-22	88	20	56
12	91	13	82	13	98	40	80	38	63
23	88	30-34	76	30-34	94				
41	76	54	53	54	84				

Germination tests were made in unsterilized soil in beds in the greenhouse, using the method described by Machacek and Wallace (6). Twenty-five seeds were sown in each row; each row was considered a "plot". Because of shortage of space, the number of replicates in individual tests varied from two to six. The tests with different seed lots were made at irregular intervals after treatment; the respective experiments were continued for from 3 to 4½ years. Preliminary stand counts were made about 2 weeks after sowing; final counts were usually made after 3 or 4 weeks. In addition to recording the number of seedlings which had emerged in each row, notes were also made of any abnormalities observed. Seedlings which damped-off were surface sterilized and plated on potato sucrose agar to determine the cause of death.

To determine the effect of treatment on seed microflora, samples of seed treated with each of the chemicals at both rates were plated on potato sucros agar in petri dishes. Check samples of untreated seeds were plated without surface sterilization, and also after surface sterilization with hypochlorite solution. In other tests, samples of seeds from each of the treatments were incubated on moist filter paper in petri dishes, and the fungi growing on the seeds were identified.

Dissection and examination of seeds from the various plating tests indicated that the microflora might be located in the hull, rather than in the "kernel". To determine this point, some seeds were plated whole as in all the other tests; other aliquots of the same samples were dehulled before plating.

RESULTS

Seedling Emergence

The results of storage on the emergence in soil of untreated sunflower seeds are given in Table 1. It is apparent that the emergence of the respective seed lots at the beginning of the storage period was not uniform. In order to simplify evaluation of the effects of the chemicals tested, the emergence of each treated sample was calculated as a percentage of the emergence of the appropriate untreated check sample for each interval of storage. The results for seed lot 3 are fairly representative; they are shown graphically in Figure 1.

Differences in emergence of seeds treated with the respective chemicals at 1½ ounces per bushel were very slight. Striking differences in the effects of the various chemicals were noted at the 15-ounce-per-bushel rate. The organic mercurial preparations – Ceresan, Leytosan, Lunasan, Panogen, and Agrox C – were consistently injurious; Semesan Bel and Mergamma C were not injurious. The non-mercurial preparations tested were not phytotoxic.

Seed lots 1 to 4 differed in their response to treatment at the heavy rate with some of the phytotoxic materials. The differences are illustrated graphically in Figure 2. As environmental conditions were not controlled in these experiments, they may have influenced the differential response to treatment of the various seed lots.

Symptoms of Phytotoxicity

The mercurial preparations, which at 15 ounces per bushel prevented or retarded emergence of sunflowers, induced characteristic symptoms of

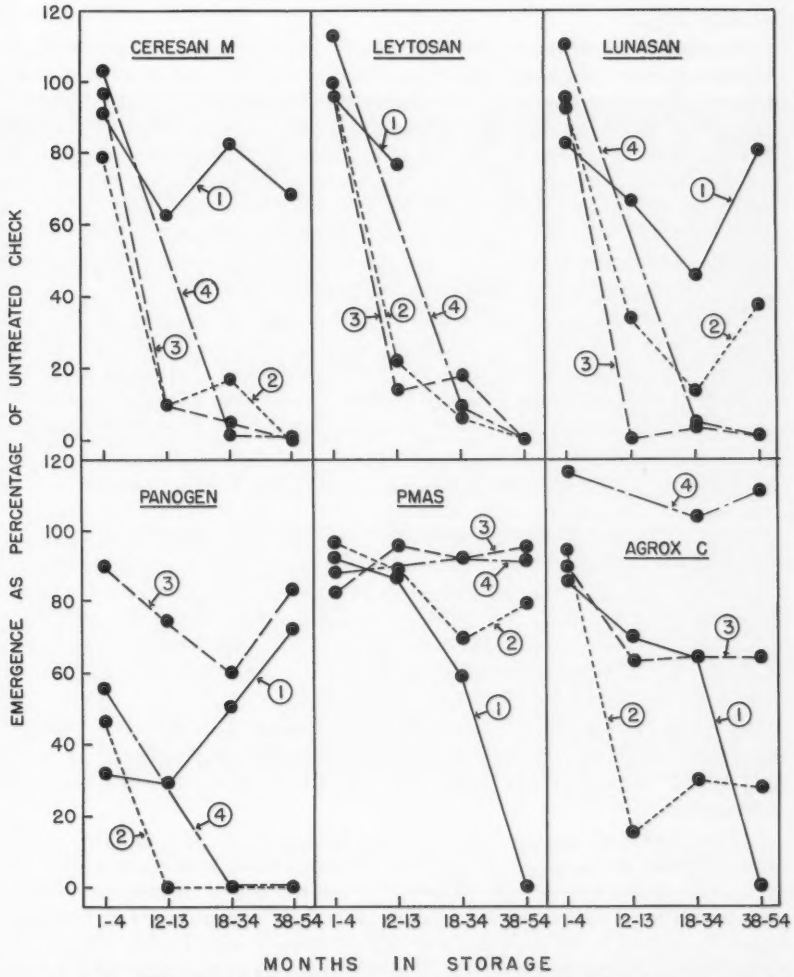

FIGURE 2. Differential effect of treatment with excessive dosage of some mercurial seed dressings and subsequent storage on emergence of four lots of sunflower seeds.

mercury poisoning on seedlings. The hypocotyls were shorter and thicker than normal, and the below-ground portion was often brown, swollen, and terminated in an acute point (Figure 3). Many seeds which failed to emerge had given rise to abortive, thick radicles which did not produce roots (Figure 4).

Non-mercurial preparations induced no adverse effects even at 15 ounces per bushel. Seedlings treated with light and heavy dosages respectively of Arasan, shown in Figure 5, are typical of all the non-mercurial treatments.

Seedling Blight

Sunflower seedlings which "damped-off" in greenhouse soil beds yielded various organisms, including *Botrytis cinerea* Pers., *Sclerotinia sclerotiorum* (Lib.) de Bary, species of *Alternaria, Aspergillus, Cephalothecium, Penicillium, Rhizopus, Stemphylium,* unidentified fungi and bacteria. As the soil used for these experiments was not sterilized, any of the organism isolated might have been soil-borne. The incidence of various kinds of seedling blight, including damping-off, was so low that no conclusions could be drawn as to the relative effectiveness of the various chemicals against the fungi involved.

Seed-borne Microflora

It has been shown by numerous investigators that cereal seeds may be killed under certain storage conditions by saprophytic fungi, as well as by known pathogens (1). Samples of the sunflower seeds used in these experiments were, therefore, plated to learn something of their microflora before treatment with fungicidal seed dressings, and after treatment to determine the effect of the various chemicals on the microflora.

Seeds treated with the various chemical seed-dressings and the untreated check samples were plated on agar without prior surface-sterilization. The seed had been stored for from 4 to 31 months after chemical treatment when the platings were made. One representative series of plates is shown in Figure 6. In spite of differences in length of storage after treatment, and differences between the original seed lots used, the results from the various treatments were similar and were averaged. The averages are given in Table 2. The only conspicuous differences between seed lots were noticed in the figures for percentage germination.

Typical symptoms of acute mercury poisoning were apparent in seed lots treated with the heavy rate of Ceresan, Leytosan, Lunasan, Panogen, and Agrox. Many of the seeds were killed, as evidenced by reduced germination.

The mercurial compounds, with the exception of Semesan Bel, were very effective in suppressing growth of fungi from sunflower seeds on agar, even at the low dosage (Table 2). At the heavy dosage they completely prevented fungal growth in most plates. The relatively few colonies which developed from seeds treated with mercurials were largely *Alternaria tenuis* Nees. *Rhizopus stolonifer* (Ehrenb. ex Fries) Lind. occurred in some plates; *Penicillium sp., Aspergillus sp., Cladosporium sp.,* and various unidentified organisms also occurred. A much higher proportion of seeds treated with Semesan Bel or with the non-mercurial preparations yielded fungus colonies, and the fungus flora was more diverse as well as more plentiful. *Botrytis cinerea* was isolated from the untreated check sample, and from samples treated with Semesan Bel or Arasan, of seed lot 1. *Sclerotinia sclerotiorum* developed from one seed of lot 5, treated with Spergon.

Some of the seeds plated in the first tests in 1952 were dissected after incubation. The fungi growing from them seemed to be restricted to the hulls and to the testa; when the testa was removed, the cotyledons appeared

Fig. 3

Fig. 4

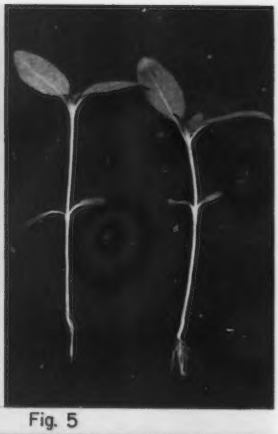

Fig. 5

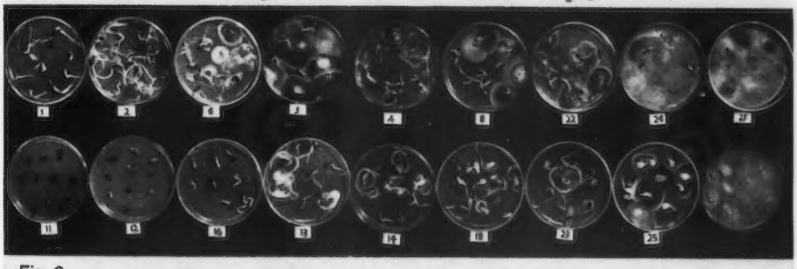

Fig. 6

FIGURE 3. Injury to sunflower seedlings caused by treatment with excessive dosage of mercurial seed dressing and storage after treatment. Seedling on *left*, 1½ ounces per bushel of Panogen 14; seedling on *right*, 15 ounces per bushel of Panogen 14.

FIGURE 4. Retardation and killing of sunflower seedlings caused by treatment with 15 ounces per bushel of mercurial seed dressing and storage after treatment.

FIGURE 5. Absence of phytotoxic effect on sunflower seedlings following treatment with non-mercurial seed dressings and subsequent storage. Seedling on *left*, 1½ ounces per bushel, seedling on *right*, 15 ounces per bushel.

FIGURE 6. Effect of treatment with chemical seed dressings and subsequent storage on microflora isolated from sunflower seeds on agar in petri dishes. *Upper row*, 1½ ounces per bushel, *lower row*, 15 ounces per bushel of chemical. 1, 11, Ceresan; 2, 12, Leytosan; 6, 16, Agrox C; 3, 13, Semesan Bel; 4, 14, Arasan; 8, 18, Spergon; 22, 23 Phygon; 24, 25, Parzate; 21, untreated check.

TABLE 2. — EFFECT OF CHEMICAL SEED TREATMENTS ON GERMINATION AND FUNGUS FLORA OF SUNFLOWER SEEDS PLANTED ON AGAR

Chemical	Dose, ounces per bushel	Germination, per cent		Clean seed, per cent		Fungi[1]		Phytotoxicity
		Av. of seed lots 1 to 4	Seed lot 5	Av. of seed lots 1 to 4	Seed lot 5	Seed lots 1 to 4	Seed lot 5	
Untreated check		90	[2]	0	0	A,B,R,U,	R	
Ceresan M	1½	94	85	99	99	U	1 U	—
	15	24	23	100	100	O	O	Severe
Leytosan	1½	95	—	89	—	P,R,U,	—	—
	15	32	—	99	—	O	—	Severe
Lunasan	1½	94	—	92	—	A,Asp.	—	—
	15	21	—	100	—	O	—	Severe
Panogen 14	1½	93	—	99	—	A	—	—
	15	21	—	99	—	O	—	Severe
PMAS	1½	93	—	93	—	A,R,U,	—	—
	15	76	—	100	—	O	—	Light
Agrox C	1½	92	—	87	—	A.R,U,	—	—
	15	55	—	99	—	O	—	Moderate
Semesan Bel	1½	92	—	52	—	A,B,P,R,U	—	—
	15	91	—	58	—	A,P,R,	—	—
Mergamma C	1½	95	—	85	—	A,C,U,	—	—
	15	93	—	100	—	O	—	—
Spergon	1½	88	92	62	49	A,Asp.,R	A,R,S,U	
	15	93	76	68	54	A,R,U	A,R,U	
Phygon	1½	90	—	68	—	A,R,U	—	
	15	86	—	53	—	A,R	—	
Parzate	1½	90	77	3	40	A,R	—	
	15	90	87	19	64	A,R,T	—	
Arasan	1½	92	94	65	0	A,R,U	100 P	
	15	94	93	71	82	A,B,U	1 U	
Thioneb 50W	1½	—	89	—	24	—	40 P,R,	
	15	—	90	—	74	—	1 U	
Captan	1½	—	81	—	56	—	R	—
	15	—	84	—	58	—	R	

[1]Fungi were: A, *Alternaria sp.*: Asp. *Aspergillus* sp.; B, *Botrytis cinerea;* C, *Cladosporium* sp.; P, *Penicillium* sp.; R, *Rhizopus stolonifer;* S, *Sclerotinia sclerotiorum;* T, *Trichoderma viride;* U, Unidentified

[2]Overrun by *Rhizopus*

free of fungus growth. In later experiments, some seeds from each sample were surface-sterilized in hypochlorite solution for 3 minutes, then plated. Other seeds were dehulled, then surface sterilized in hypochlorite solution for 1 minute and plated. Fifty-nine per cent of the whole seeds remained clean; thirty-one per cent gave rise to *Alternaria*, two per cent to unidentified fungi, and four per cent to bacteria. Ninety-eight per cent of the dehulled seed remained clean; two per cent gave rise to bacteria. In other tests, occasional fungus colonies grew from dehulled kernels.

The plating results and examination of dissected seeds indicate that most of the microflora of sunflower seeds is confined to the hull; it may be external, on the inner surface, or within the tissues of the hull. As even very light surface sterilization practically eliminates *Rhizopus*, this organism is apparently usually borne superficially. *Alternaria* sometimes

survives very severe surface sterilization; it apparently can penetrate deep into the hull tissues. Some micro-organisms also develop on or in the surface tissues of the testa. Very light surface sterilization of dehulled seeds usually destroys most of the fungi, although in occasional samples the testa tissues appear to be penetrated more deeply.

DISCUSSION

From the results presented above, it is apparent that the germinability of sunflower seeds remains fairly high in storage for 2 to 3 years, but may decrease appreciably if the seed is stored longer than 3 years.

Treatment with the non-mercurial seed dressings tested in these experiments had no adverse effect on seed germination after prolonged storage, even when the chemicals were applied in excess. Mercurial seed dressings applied at 1½ ounces per bushel also had no adverse effect on germination. When applied at 15 ounces per bushel, some of the mercurials were highly phytotoxic, particularly if the seed was stored for 2 years or more after treatment.

Farmers who treat their seed before sowing rarely store the treated seed longer than a few months; even if they fail to sow it in the spring, they rarely have to store it longer than a year. None of the chemicals in these tests, applied at the rate of 1½ ounces per bushel, injured germination after a year or even longer periods of storage.

Suppliers of foundation grade seed for the production of hybrid sunflower varieties often produce such seed only once in several years, then store it until the supply is exhausted. If seed treatment helps to retain germinability of stored seed at high levels for long periods, it would be advantageous to treat with the appropriate chemicals to protect the seed from injury by fungi in storage.

The mercurial fungicides appeared to be most effective of those tested in inhibiting the growth of fungi from the seeds. They were effective at the rate of 1½ ounces per bushel, which did not affect seed germination adversely even after long periods of storage. The fungi which grew out from seed treated with non-mercurial preparations apparently had very little deleterious effect, as such seed lots germinated as well as those treated with the mercurials. The germinability after storage of some samples of seed treated with the low dosage of mercurials and both low and high dosage of non-mercurials was higher than that of untreated check samples. The reason for the slight protectant action in storage of these seed dressings was not determined, but might be associated with the reduction in numbers of some components of the microflora.

Although the significance of most of the seed microflora was not determined, its source is fairly obvious. Sunflower growers in Manitoba usually delay harvesting until severe fall frosts kill the plants, making combine harvesting easier. Weather conditions in the fall are often very favourable for development of various fungi on sunflower heads, which appear to be an excellent substrate for mould growth. The high degree of fungus infection of the seeds which normally occurs is rarely visible to the naked eye although, as shown in this paper, it is very readily demonstrated by plating.

The results reported here indicate that most chemical seed dressings can be applied, at rates commonly recommended for other seeds, to sunflowers without causing any injury in the absence of seed-borne pathogenic fungi. It still remains to be shown which seed dressings, if any, will control seed-borne *Verticillium albo-atrum*, and if any available seed treatment chemicals will protect sunflowers from attack by such soil-borne pathogens as *V. albo-atrum*, *Sclerotinia sclerotiorum*, and *Plasmopara halstedii*.

ACKNOWLEDGEMENT

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ATMOMETER ASSEMBLIES, A COMPARISON[1]

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ABSTRACT

The field performance of six different atmometer assemblies was determined during the growing seasons of 1956 to 1959. The black Bellani plate atmometer equipped with Livingston-Thone wool mercury valve was used as standard. Other black Bellani assemblies involved Shive's mercury U-tube valve, fritted glass valve, and two different mounts using plastic shields instead of valves. An assembly functioning on the principle of a Piché atmometer and using a black porous disc as absorbent pad was also tested.

The daily response of all valve-equipped atmometers to changing meteorological conditions was essentially the same. Since the U-tube and fritted glass valves were free from blockage by foreign matter, assemblies having them proved superior to the standard. However, these valve types did not solve the difficulty of priming. The use of shields to avoid this difficulty increased the evaporation rate and atmometers so equipped responded significantly differently from the standard. The black porous disc assembly also lost more water by evaporation than the standard but its response was not significantly different from the conventional assembly. Since the black porous disc mount has neither valve nor shield it is easier to maintain and operate than the other assemblies. It proved also less susceptible to frost damage.

INTRODUCTION

The black Bellani plate atmometer is considered by some workers (1, 7, 10) as a superior instrument for the measurement of evaporation, particularly for obtaining an estimate of water lost from crop plants. No instrument of like simplicity so closely approximates the integration of the influences of sunshine, wind, temperature and atmospheric humidity on evaporation to that of a plant.

The Bellani plate atmometer (3, 5, 7) consists of a flat, circular evaporating surface of porous porcelain covering the top of a glazed porcelain funnel (Figure 1A). Water is conducted to the funnel from a burette which acts as a reservoir and measuring device (Figure 2A). As usually assembled, a mercury and wool valve (Figure 1B) is placed in the conducting tube from the reservoir to the funnel. This prevents water from backing up into the reservoir when rain falls or dew forms on the porous plate.

As pointed out by Robertson and Holmes (9), the black Bellani plate atmometer has many advantages. It is simple, easy to operate and to maintain. It is small, easily transported and its parts are inexpensive and commercially available. However, the instrument is susceptible to frost damage and is somewhat difficult to prime, i.e., establish an air-free column of water from the reservoir to the evaporating surface. The wool mercury valve, besides increasing the difficulty of priming, is subject to clogging.

The object of this investigation was to find or develop an atmometer as free as possible from the disadvantages of the wool mercury valve and not to introduce an instrument immune to frost damage. Nevertheless, as will be pointed out later, an atmometer type was tested which proved less susceptible to frost damage than the Bellani plates.

[1]Contribution from the Research Branch, Canada Department of Agriculture.
[2]Agronomist.

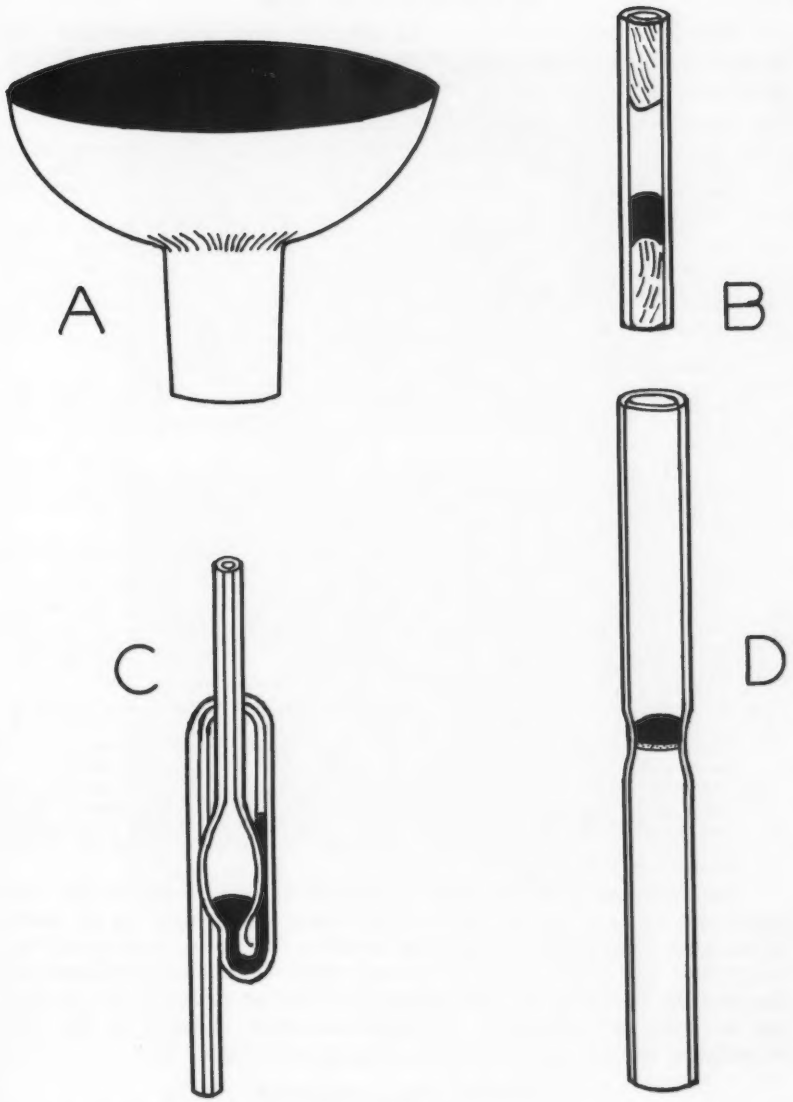

FIGURE 1. A. Black Bellani plate atmometer.
B. Livingston-Thone wool mercury valve (mercury represented in black in all valves).
C. Modified Shive mercury U-tube valve.
D. Fritted glass valve.

MATERIALS AND METHODS

The daily evaporation from six different atmometer assemblies was compared during the summers of 1956 to 1959. Five used the black Bellani plate as an evaporating surface, the sixth a black porous disc (Figure 2D).

Atmometer assemblies under test were:

1. *The "Standard"*. This consists of a black Bellani plate with Livingston-Thone wool mercury valve (Figure 2A). This valve, in general use since 1920 (3), consists of a short glass tube containing a small quantity of mercury held between wool plugs (Figure 1B).

   An undesirable feature of the wool mercury valve is that the wool traps impurities in the water which in time is prevented from flowing freely to the evaporating surface. The use of distilled water does not entirely overcome this difficulty as algae and fungi lodge in the wool plugs and multiply. These microflora can be discouraged by use of disinfectants (3, 6, 8). Blockage can be caused also by the wool strands breaking the mercury into very minute beads which choke the capillaries of the plug. Vibration of the apparatus by wind induces this condition.
2. *The "Mercury U-tube Valve"*. In this mount the valve and conduit to the atmometer plate are contained within the reservoir (Figures 1C, 2B). The U-tube valve was introduced by Shive (11) and with modification has been used since with success (12).
3. *The "Fritted Glass Valve"*. Assemblies using this valve are identical to the standard except that the wool mercury valve is replaced by a fritted glass valve (Figure 1D). Atmometers equipped with valves of fritted glass were first employed by Robertson and Holmes (9).
4. *The "Shielded Plastic Mount"*. This assembly which also uses a black Bellani plate as an evaporating surface, was designed to eliminate the need of a valve (8) (Figure 2C). The shield, of plastic like the reservoir, is held directly above the atmometer head to prevent rain from falling upon the evaporating surface. Near the perimeter of the underside of the shield is a groove to prevent drip accumulating at the centre of the shield and falling upon the atmometer plate. Because this assembly was not designed for daily records its reservoir is large. This reduces the accuracy and ease with which readings may be taken.
5. *The "Shielded Burette Mount"*. This assembly is so constructed that errors in reading because of parallax do not occur as they do with the plastic mount. A burette of the same dimensions as that used in the standard mount but with its lower end sealed takes the place of the plastic reservoir (Figure 3B and F).
6. *The "Black Porous Disc"*. This assembly was designed* to avoid the need of valve or shield (Figure 2D). It consists of a column of water held above a black porous absorbent pad. The pad or disc is made of "Alundum" a crystalline aluminous abrasive. The assembly functions on the principle of the Piché atmometer (2, 4).

The different assemblies were established in pairs to ensure that one instrument of each pair would be functioning satisfactorily at all times. When both instruments of a pair functioned normally, i.e., their water loss was similar and seemingly in consonance with the ambient conditions of the previous 24 hours, the reading from the one that measured the greatest loss of water was recorded. All assemblies were mounted so that the evaporating surface was 4 feet above the ground (Figure 3).

RESULTS AND DISCUSSION

The evaporation from the standard, the U-tube valve and the fritted glass valve atmometer assemblies was very similar (Tables 1, 2, 3). Since the two latter have valves that are not subject to blocking, they are superior to the standard.

*Robertson, G. W., Division of Field Husbandry, Soils and Agricultural Engineering, Central Experimental Farm, Ottawa, Ont. *Personal communication.* 1957.

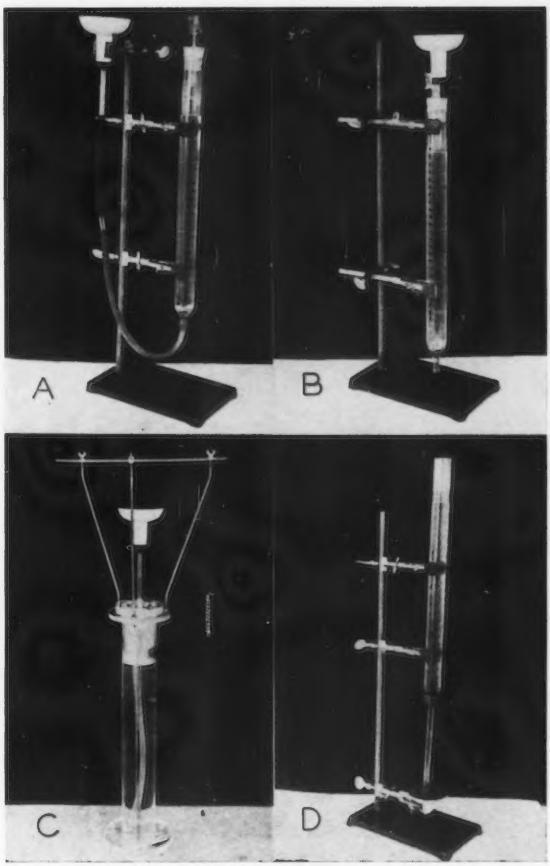

FIGURE 2. Different atmometer assemblies:

A. Standard wool mercury valve.

B. Mercury U-tube valve.

C. Shielded plastic mount.

D. Black porous disc.

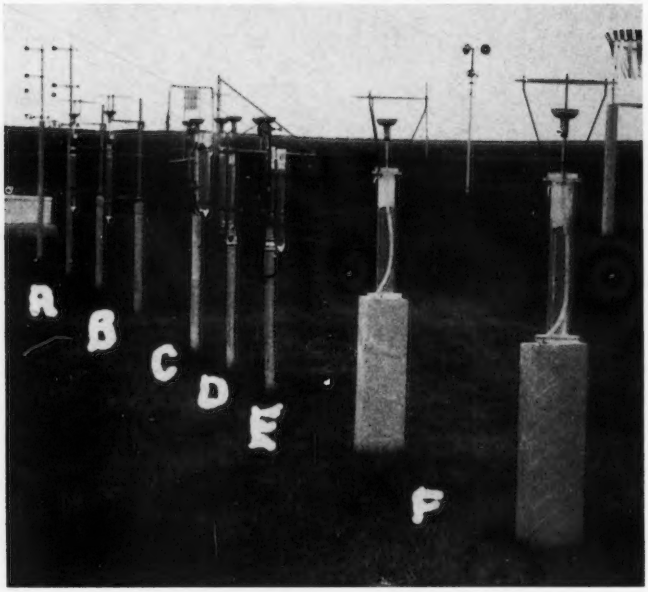

FIGURE 3. Atmometer assemblies under comparison:

A. Black porous disc.
B. Shielded burette mount.
C. Fritted glass valve.
D. Mercury U-tube valve.
E. Standard wool mercury valve.
F. Shielded plastic mount.

TABLE 1. — RELATIVE EVAPORATION FROM DIFFERENT ATMOMETER ASSEMBLIES UNDER DIFFERENT WEATHER CONDITIONS

Assembly type	All days	Days without rain	Days with rain	Days with trace of rain[1]
Standard	100.00	100.0	100.0	100.0
U-tube valve	101.8(277)[2]	101.8(167)	101.8(93)	101.1(17)
Fritted glass valve	100.4(185)	100.2(118)	101.4(58)	100.5(9)
Shielded plastic mount	108.5(407)	106.5(240)	113.0(148)	114.8(19)
Shielded burette mount	103.7(171)	102.3(98)	107.6(71)	103.1(2)
Porous disc	105.8(230)	106.2(133)	104.9(89)	107.3(8)

[1]Less than 0.01 in. of rain
[2]No. of days compared

TABLE 2. — CORRELATION COEFFICIENTS BETWEEN DAILY EVAPORATION FROM DIFFERENT ATMOMETER ASSEMBLIES

Assemblies correlated	Correlation coefficient r	No. of days n	1% sig.
Standard — U-tube valve	.997	277	.154
Standard — Fritted glass valve	.997	185	.188
Standard — Shielded plastic mount	.972	407	.127
Standard — Shielded burette mount	.995	171	.194
Standard — Porous disc	.988	230	.169

Apparently there is little to choose between the U-tube valve and the fritted glass valve assemblies. The valves of both can be obtained commercially; the U-tube valve can actually be made in the laboratory. The U-tube valve ensures compactness but makes the assembly somewhat more difficult to prime than the standard or fritted glass valve mounts. Moreover, readings taken from the reservoir of the U-tube valve assembly must be adjusted to compensate for water displacement by the conduction tube.

The data of Table 1 show that the replacing of valves by shields increased the evaporation rate. For all days of the test the increase was 8.5 per cent for the plastic mount and 3.7 per cent for the burette assembly. Because of errors of parallax associated with the plastic mount, records from the burette mount are stressed in the comparison of the valved and shielded mounts. As indicated, the difference is slight. When shields are used valve troubles are eliminated and the instrument becomes easier to prime. However, shields need frequent cleaning to reduce radiation effects to a minimum.

It is difficult to point out why shielded mounts should lose more water by evaporation than the standard assembly. It may be because valved assemblies imbibe minute quantities of water during times of rain, since the mercury bead moves slightly depending upon the direction of the water flow or pressure. That this occurs is indicated by the data in Table 1 which show that the greatest difference between shielded and unshielded atmometers occurs during times of rain. The shielding of the atmometer might also change the heat balance of the instrument, not only because of

TABLE 3. — COMPARISON OF DAILY EVAPORATION FROM DIFFERENT ATMOMETER ASSEMBLIES OVER A 40-DAY PERIOD, AUGUST 12–SEPTEMBER 20, 1958

Assemblies compared	Value of t	P.05	P.01
Standard — U-tube valve	0.68	2.02	2.70
Standard — Fritted glass valve	1.03	2.02	2.70
Standard — Shielded plastic mount	2.40	2.02	2.70
Standard — Shielded burette mount	2.02	2.02	2.70
Standard — Porous disc	1.11	2.02	2.70

the effect of the shield on the incoming total solar radiation but on the long wave radiation received from the surface of the earth and reflected by the shield on to the instrument.

Data in Table 1 also show that there is a definite differential response to varying weather conditions between the shielded plastic mount and the standard. Why rate of evaporation from the plastic mount during days with only a trace of rain was so much greater than those of the other assemblies under similar circumstances is difficult to say, unless it is that on such days the shields radiate and reflect the long-wave energy from the ground back on to the atmometer plate, thus increasing the amount of energy available for evaporation. The data show that this condition does not hold with the shielded burette mount, but here the number of days with a trace of rain are too few to provide a test.

Although there are no significant differences between the correlation coefficients presented in Table 2, the fact remains that the correlation between the response of the shielded burette mount and the standard to varying weather conditions is closer than between the shielded plastic mount and the standard. Table 3, comparing the daily response of the different atmometers for a shorter period of time, substantiates this relationship. The data show that the responses of the shielded plastic mount and the standard are significantly different, while the difference between the responses of the shielded burette mount and standard just reach the level of significance.

Throughout the test the black porous disc atmometer lost 5.8 per cent more water than the standard (Table 1). This greater evaporation from the disc is difficult to explain. Since the water loss from the disc was very similar to that from the standard on both rainy days and days without rain, the contention that small quantities of water over and above the absorbing capacity of the disc are forced out on to it by the air in the reservoir above the water column expanding on being warmed by the sun does not hold. In these tests, attempts were made to keep undue leakage of water upon the disc to a minimum by keeping the air column small by daily filling of the reservoir. A more logical explanation for the greater evaporation from the porous disc is that the water on its surface is under no tension since it is virtually flooded at all times, while the water at the surface of the Bellani plate is under slight tension due to capillary action. Further, the surface area of the porous disc whence water of the reservoir is evaporated may be a little larger than that of the Bellani plate.

Despite the fact that slightly more water is evaporated from the porous disc than from the standard, the response of these two assemblies to changing meteorological conditions is close (Table 2). Moreover, the short-term test (Table 3) shows no significent difference between the response of the two instruments.

The porous disc apparatus has several advantages over the Bellani assemblies. It is easier to prime, particularly more so than the valved types. No coefficient is required to bring the responses of several discs to a common basis as is necessary with Bellani plates. Moreover, the disc is better able to withstand the action of frost than the Bellani plate.

SUMMARY

A disadvantage of the conventional atmometer mount is the valve. Attempts to improve upon the standard wool mercury valve brought forth the U-tube and the fritted glass types. Although these proved free from contamination by impurities in the water, they did not solve the difficulty of priming.

Priming difficulties were reduced by using rain shields in place of valves. However, shields have to be kept clean. A shielded assembly using a burette reservoir lost 3.7 per cent more water by evaporation over a period of 171 days than the standard valved mount. Correlation of the response of the two types to varying weather conditions was highly significant, .995 (P.01 = .194).

A Piché-like atmometer, using a black porous disc as an evaporating surface, lost 5.8 per cent more water by evaporation over a period of 230 days than the standard mount. Correlation with the standard was highly significant, .988 (P.01 = .169). Except for the slightly greater rate of evaporation from the porous disc than from the Bellani plate these two assemblies performed similarly. The porous disc apparatus, however, proved to be a superior instrument as it has no valve to become contaminated, does not require use of a correction coefficient, is easy to prime and is less susceptible to frost damage.

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INSECT TRANSMISSION OF THE VIRUS CAUSING LITTLE CHERRY DISEASE[1]

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ABSTRACT

Little cherry virus disease of sweet cherry (*Prunus avium* L.) was transmitted under screenhouse conditions by 3 species of leafhoppers (*Homoptera*: *Cicadellidae*) out of 24 species tested. *Macrosteles fascifrons* (Stal), the 6-spotted leafhopper, transmitted the disease in seven tests; *Scaphytopius acutus* (Say), the sharp-nosed leafhopper, transmitted it once; and *Psammotettix lividellus* (Zett.) transmitted it once. The transmissions were made from diseased sweet cherry trees of the variety Lambert to indicators of the varieties Star or Sam. With the exception of 1 transmission, 2 to 4 years were necessary following inoculation for unmistakable expression of symptoms in the indicators. *M. fascifrons* was also implicated in 18 successful transmissions to mature sweet cherry trees grown in the open.

INTRODUCTION

Little cherry disease of sweet cherry was first found in 1933 (3) at Willow Point on the west arm of Kootenay Lake, British Columbia. It spread rapidly to all the orchard areas around Kootenay Lake and has been the major factor in the decline of cherry production in these areas. In 1941 (4) it was shown that the symptoms could be reproduced by budding from diseased to healthy trees, and it was concluded that the disease was caused by a virus. The fruit from affected trees is reduced in size, pale in colour, and insipid or bitter in taste.

Recent investigations have indicated that the virus of little cherry disease of sweet cherry is identical with a latent virus found in Kwanzan and Shirofugen Japanese flowering cherries throughout North America (11) which caused the so-called K. & S. disease. Western X little cherry also causes small fruit symptoms in cherry (10), but differences in the host ranges suggest that these are separate diseases.

Although it is not yet found in British Columbia outside the Kootenay area, little cherry disease remains a potential threat to cherry plantings in the province, most of which are in the Okanagan Valley.

This paper describes work which has been in progress since 1950 at Creston, British Columbia, in attempts to find the vector or vectors responsible for spreading the disease.

MATERIALS AND METHODS

Since 1933, when Kunkel (7) demonstrated that the leafhopper *Macropsis trimaculata* F. was a vector of peach yellows disease virus, leafhopper vectors have been found for other stone fruit virus diseases (1, 4, 5, 6, 13, 14, 20, 21), so that there was reason for regarding leafhoppers as prime suspects. Early transmission results indicated that leafhoppers were capable of spreading little cherry. Table 1 lists the insects and mites, field collected or laboratory reared, tested as possible vectors of little cherry virus during the years 1950-1957. The systematic arrangement and nomenclature in the leafhoppers are those of Beirne (2) and Oman (8). The insects used in the tests were identified by the Systematic Unit, Entomology

[1]Contribution No. 31, Research Station, Research Branch, Canada Department of Agriculture, Summerland, B.C.

[2]Part of a thesis submitted to the Graduate School, Utah State University, Logan, Utah, June 1958, in partial fulfilment of the requirements of the degree of Doctor of Philosophy.

TABLE 1. — INSECTS AND MITES TESTED AS POSSIBLE VECTORS OF LITTLE CHERRY VIRUS CRESTON, B.C., 1950-1959

INSECTS	
ORDER HOMOPTERA	
Family Cicadellidae	
Subfamily Idiocerinae	
Tribe Idiocerini	
	Idiocerus populi (L.).
	Idiocerus provancheri Van D.
	Idiocerus musteus Ball
Subfamily Iassinae	
Tribe Gyponini	
	Gyponana angulata Spang.
Subfamily Aphrodinae	
Tribe Aphrodini	
	Aphrodes costata (Panz.)
	Aphrodes flavostrigata (Don.) = *albifrons* Ball
Subfamily Neocoelidinae	
Tribe Neocoelidini	
	Stenocoelidia lineata (Bak.)
Subfamily Tettigellinae	
Tribe Tettigellini	
	Neokolla hieroglyphica (Say)
Subfamily Typhlocybinae	
Tribe Erythroneurini	
	Erythroneura aspera B. & G.
	Erythroneura dolosa B. & G.
	Erythroneura plena Beamer
Tribe Typhlocybini	
	Typhlocyba pomaria McA.
	Typhlocyba gillettei (Uhl.)
	Edwardsiana rosae (L.)
	Empoasca maligna (Walsh)
Subfamily Deltocephalinae	
Tribe Scaphytopini	
	Scaphytopius acutus (Say)
Tribe Balcluthini	
	Balclutha punctata (Thumb.)
Tribe Macrostelini	
	Macrosteles fascifrons Stal.
Tribe Deltocephalini	
	Osbornellus borealis Del and Mohr.
	Colladonus geminatus Van D.
	Colladonus montanus Van D.
	Euscelidius schenki (Kirsch.)
	Exitianus exitiosus (Unl.)
	Psammotettix lividellus (Zett.) = (*affinis* Gill. & Bak.)
Family Aphidae	*Myzus cerasi* (F.)
Family Psyllidae	*Psylla trimaculata* Var. *astigmata* (C.)
Family Membracidae	*Ceresa basalis* Walk.
	Stictocephala pacifica Van D.
Family Miridae	*Capsus ater* (S.)
	Stenotus binotatus (F.)
	Lygus hesperus K.
Family Tingidae	*Corythuca mollicula* O. & D.
Family Cercopidae	*Philaenus leucophthalmus* (L.)
MITES	
ORDER ACARINA	
Family Tetranychidae	*Panonychus ulmi* (Koch.)
	Tetranychus bimaculatus occidentalis Dillon
	Tetranychus mcdanieli McGregor
	Bryobia praetiosa Koch
Family Eriophyidae	*Diptacus* sp.
	Vasates sp.

Division, Canada Department of Agriculture, Ottawa, Ontario. Tetranychid and Eriophyid mites were identified by C. V. G. Morgan, Entomology Laboratory, Canada Department of Agriculture, Summerland, British Columbia.

Straight-flow aspirators (9) were operated in the laboratory by a vacuum pump, and in the field by adapting the available vacuum from the engine of a stock ½-ton pick-up truck (18). Mechanically operated aspirators made it possible to collect, segregate, and place on test trees hundreds of insects in a short time. The use of a steady, controlled vacuum of 2 to 5 inches Hg prevented damage to the insects.

The use of bi-valve cages on unscreened or screened trees (17) reduced losses of test insects, provided protection against wind and weather, facilitated placing and removing insects, and was adaptable to seedling and mature cherry trees.

Single, mature test trees of the varieties Bing or Lambert were used in the open, or enclosed in individual screenhouses during the first years of the investigation. After 1953, 2- and 3-year-old leaf-indicator trees of the varieties Sam, Star, or S-8-4 were used (19) under screenhouse conditions. Three or more insecticide sprays were applied each season to reduce thrips and mites, which were capable of penetrating the fine 32-mesh per inch Saran screen of the screenhouses, and to reduce arthropod populations in unscreened test plots.

Routine vector testing consisted of making large collections of leafhoppers or other possible vectors, establishing these in holding cages containing grasses and legumes, then giving them an acquisition feed of 2 to 48 hours on diseased cherry foliage. From 1947 to 1954 they were transferred directly from the diseased foliage to the indicators; but from 1955 on, they were given a latent period of from 20 to 30 days on preferred hosts, usually fall rye or barley (12), which are believed immune from any known stone fruit virus. Then the vectors were segregated to species whenever possible, and from 2 to 104 specimens placed in bi-valve cages on the indicators. The latent period on rye or barley was provided because sweet cherry is not a preferred host for most of the species tested.

RESULTS AND DISCUSSION

In the course of the early attempted transmissions to unscreened trees, consistent ability to transmit was shown by *Macrosteles fascifrons* (Stal.), which was implicated in 18 successful transmissions to isolated mature sweet cherry trees planted in the open. Because of the possibility that natural spread might have interfered with all but 2 of these transmission results,

TABLE 2. — TRANSMISSION OF LITTLE CHERRY VIRUS DISEASE TO SCREENED INDICATOR TREES BY *Macrosteles fascifrons* (STAL.), ROBSON AND CRESTON, B.C., 1953-1959

Year	1953	1954	1955	1956	1957	1958	1959	Totals
Tests	11	43	20	36	36	192	110	448
No. of insects used	97	511	224	720	324	1728	1650	5254
Trees inoculated	6	16	6	13	56	34	59	190
Trees infected	1	2	2	0	1	1	—	7

M. fascifrons was not confirmed as a vector until results from screened trees became available (Table 2, 3).

Experimental transmissions to test Star or Sam trees have shown intense colour symptoms on foliage in the second, third or fourth years. By contrast, budding or grafting with diseased material produces severe systemic shock and intense colour reaction on foliage after one year, or, if the budding is done early in the season, even in the same year.

The symptoms in Star or Sam indicators infected by insects or budding were not affected by the degree of light reduction experienced in the screenhouse, although greatly reduced light modifies the severity of the symptoms in mature Lambert fruits (16).

Symptoms indicating that little cherry disease had been transmitted were verified by co-operating plant pathologists. For each series of test trees, checks were provided at the rate of one check to every 5 test trees in the open, and one check to every 7 test trees in the screenhouses. None of the checks in unscreened tests showed any symptoms of little cherry disease during the investigational period from 1950-1954. None of the 30 check trees interplanted with the 212 Star or Sam in screenhouses showed any little cherry symptoms after 6 years of test insect investigations had been completed. Because 5 of the 30 check trees were growing at a distance less than 3 feet from little cherry disease inoculated test trees, and because foliage and limbs of check and diseased trees intermingled in the screenhouses, it is highly probable that mite or thrip migrations between these trees would have caused little cherry disease to spread into check trees had these arthropods been vectors.

Once test insect placement in bi-valve cages on screenhouse Star or Sam trees began, insecticide spraying for control of thrips and mites in the screenhouses was discontinued. This procedure prevented mortality of test insects from spray residues. Occasional mite and thrip population build-ups in screenhouses resulted from curtailment of these control sprays.

In screenhouse tests (Table 3), the leafhoppers, *Scaphytopius acutus* (Say) and *Psammotettix lividellus* (Zett.) = (*affinis* G. & B.), each transmitted little cherry disease once. Single transmissions indicate that further work should be done with these species.

Data suggest that transmissions are likely to occur at any time during the summer.

External and uncontrollable factors which interfered with test work were: extreme winter temperatures which killed test trees; bears which damaged trees and cages; theft of ripening and diagnostic fruits; and indiscriminate or accidental spraying by growers in test plots.

M. fascifrons counts from sticky board traps located in cover crop and sweet cherry tree canopies indicate two population peaks occur annually in Kootenay Valley, the first in mid-June, and the second in August*. The second peak shows the greatest numbers and is of longest duration. Large numbers of leafhoppers from the late season population can be collected from sweet cherry foliage.

*Wilde, W. H. A., and H. R. MacCarthy. A survey of flying Homoptera and thrips in a Kootenay cherry orchard. *Unpublished.*

TABLE 3. — DETAILS OF TRANSMISSIONS OF LITTLE CHERRY DISEASE BY LEAFHOPPERS TO SCREENED TREES, CRESTON, B.C., 1953-1959

Vector	Number used	Source	Test period	Indicator	Appearance of definite symptoms
M. fascifrons	20	Cover crop	June 23, 1954–Sept. 16, 1954	Star	July 1956
M. fascifrons	16	Cover crop	Aug. 11, 1953–Sept. 26, 1953	Star	Aug. 1957
M. fascifrons	30	Sweet cherry	Sept. 3, 1953–Sept. 29, 1953	Lambert	Aug. 1957
M. fascifrons	29	Cover crop	Aug. 2, 1954–Oct. 18, 1954	Star	Aug. 1958
M. fascifrons	17	Cover crop	June 25, 1954–Oct. 15, 1954	Star	Sept. 1958
M. fascifrons	30	Cover crop	June 24, 1958–July 15, 1958	Sam	Aug. 1959
M. fascifrons	20	Cover crop	June 17, 1957–Sept. 10, 1957	Sam	Aug. 1959
Scaphytopius acutus	2	Sweet cherry	Aug. 4, 1954–Oct. 14, 1954	Star	July 1957
Psammotettix affinis	37	Cover crop	June 29, 1954–Sept. 15, 1954	Star	Aug. 1957

Large collections of *M. fascifrons* taken in the field do not appear to contain many infectives (Table 2), but the enormous numbers of this leafhopper normally found in orchards in mid- and late-summer might account for the rapid spread of the disease within and between orchards.

M. fascifrons can feed and survive on sweet cherry in screenhouses for as long as 45 days, provided that temperature and relative humidity are not extreme. Nymphs were never found and it was assumed that few, if any, developed on sweet cherry (15). Nymphs did not survive more than 4 days on sweet cherry in cages.

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REACTION OF BARLEY VARIETIES AND SELECTIONS TO ROOT ROT AND SEEDLING BLIGHT INCITED BY *HELMINTHOSPORIUM SATIVUM* P.K. AND B.[1]

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ABSTRACT

A satisfactory inoculation technique for testing the reaction of barley varieties and selections to root rot and seedling blight incited by *Helminthosporium sativum* was developed. Surface-sterilized seeds were grown in a planting mixture of 90 per cent sterilized white silica sand and 10 per cent sand-cornmeal inoculum of the Ottawa isolate of the fungus. Disease ratings of the seedlings were recorded after 21 days in a glass-enclosed chamber kept at a temperature of 20-22°C. and a high humidity. A total of 600 varieties and selections were tested in this study. Fifty-one were classified as resistant and, of these, Anoidium, Br.3962-4, Lenta and Opal B were rated the highest. One hundred and forty-two entries were classified as susceptible. The remaining entries were intermediate in reaction.

INTRODUCTION

Helminthosporium sativum P.K. and B. (*H. sorokinianum* Sacc. ex Sorokin (9)) (*Bipolaris sorokiniana* (Sacc. in Sorok.) Shoemaker (10)) is an important pathogen in most cereal-growing areas. This fungus attacks barley, wheat, oats and a number of grasses. The various phases of the disease, root rot and seedling blight, spot blotch and head blight are responsible for a continuing loss in crop yield. Infection can be initiated by conidia or mycelium at any stage in the growth of the plant. Root rot and seedling blight are often thought to be caused by a complex of fungi but it is generally agreed that *H. sativum* is the most important component.

The production of resistant varieties appears to be the only practical means of controlling this disease. Hayes and Stakman (5), Hayes *et al.* (6), Griffee (3), and, more recently, Arny (2), studied the inheritance of reaction to spot blotch incited by *H. sativum*. They reported that different varieties and hybrids exhibited varying degrees of resistance to attack by this fungus. Wood *et al.* (11) subjected numerous varieties and hybrid lines of barley to artificial epidemics of Helminthosporium blight and found that most were susceptible. The most resistant types were selections from "Manchuria" and "Brandon", as well as other hybrid lines from diverse crosses. Hamilton and Clark (4) reported that the barley varieties Anoidium, Rabat, Prospect, Velvon 11, Comfort and Br.3962-4 revealed a high degree of tolerance to one isolate of the organism.

Breeding programs have been hampered in the past because satisfactory inoculation techniques for selecting resistant material were not available. A technique developed by Ludwig *et al.* (8) at Macdonald College for testing the reaction of barley to root rot and seedling blight showed promise as a method of screening for resistance. In 1950, the Cereal Crops and Botany and Plant Pathology Divisions, Ottawa, initiated a co-operative project to test barley varieties and hybrids for resistance to root rot and seedling blight caused by *H. sativum* under greenhouse conditions. This paper summarizes the work on the modifications of the above technique as a means of screening for resistance and the results obtained to date from the testing program.

[1]Contribution No. 31 from the Genetics and Plant Breeding Research Institute, Research Branch, Canada Department of Agriculture, Ottawa, Ont.

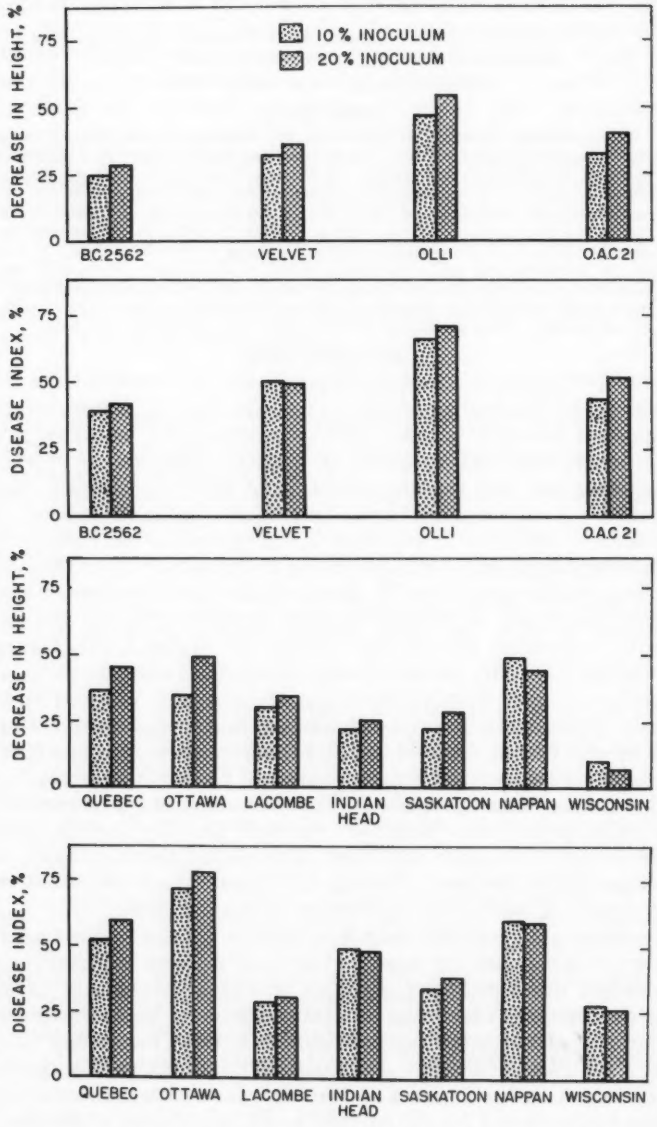

FIGURE 1. Average disease reactions of four barley varieties to seven isolates of *H. sativum* and average virulence of the seven isolates on the same varieties, using two rates of inoculum, expressed by per cent disease index and per cent decrease in height of infected seedlings. (B.C. 2562 = Rabat).

MATERIALS AND METHODS

Inoculum for the root rot and seedling blight investigations was produced by growing the fungus on a sand-cornmeal-nutrient salt medium (8). The medium was prepared by thoroughly mixing one part by weight cornmeal with 20 parts, clean, dry white sand. The mixture was dispensed into 500-ml. wide-mouth Erlenmeyer flasks, approximately 200 grams per flask, moistened with a nutrient solution made from inorganic salts in Czapek's medium (7) and sterilized by autoclaving. Each flask was then inoculated with one millilitre of a concentrated aqueous mycelial and spore suspension of *H. sativum* prepared from 7-10 day old slant cultures and incubated for 10 days at 24°C. This method gave a more uniform infection of susceptible plants than that secured by infesting the seed or soil with either spores or mycelium of the fungus. In addition, the inoculum was easier to handle and could be mixed uniformly with the planting medium.

The plants under test were grown in a mixture of a sterilized planting medium and sand-cornmeal inoculum. The inoculum was first sieved through a ⅛-inch mesh screen to obtain a uniform aggregate. The planting medium and inoculum were mixed with a mechanical beater-type mixer. At first, greenhouse soil was used as the planting medium and the infestation level was varied from 0 to 30 per cent inoculum. Later, the tests were standardized, using a light sandy soil as the planting medium and 30 per cent inoculum. The final and best modification was to mix 10 per cent inoculum with 90 per cent white silica sand.

Monoconidial isolates of the fungus used in this study were obtained from the following areas: Macdonald College, Quebec; Ottawa, Ontario; Nappan, Nova Scotia; Indian Head, Saskatchewan; Saskatoon, Saskatchewan; Lacombe, Alberta; and Madison, Wisconsin. All isolates were maintained on potato-dextrose agar.

The seeds were sized and only those which remained on top of a 5/64 x ¾-inch screen were used. The test samples were surface sterilized in a 2 per cent solution of chlorine and allowed to dry for 24 hours before planting. Four replicates were used for each entry, 25 kernels per replicate. Standard check varieties were grown in uninfested sterilized greenhouse soil for comparative purposes.

The kernels were planted in plant bands 3 x 3 x 3 inches in size, using one band per replicate. The plant bands were placed in small wooden flats, four bands to a flat. Equal amounts, by volume, of the planting medium and inoculum were placed in the plant bands and uniformly tamped. The kernels were placed in the centre of the band, covered to a depth of 1 inch with planting medium and inoculum and lightly tamped again. All treatments were watered uniformly at the beginning of each experiment and only as necessary thereafter. The flats were then placed for 21 days in glass-enclosed chambers in which the temperature and humidity were controlled. The temperature in the chambers ranged from 20-22°C. and the humidity was maintained at a uniformly high level.

TABLE 1. — SEEDLING REACTION OF BARLEY VARIETIES AND ADVANCED SELECTIONS WHEN GROWN IN A MEDIUM INFESTED WITH THE OTTAWA ISOLATE OF *H. sativum*

Resistant	Intermediate			Susceptible
Anoidium, C.I.7269*	Barboff	Glabron, C.I.4577	Plush	Canadian Thorpe
Australian, C.I.3038	Bargiers	Heimdal, C.I.8094	Proctor	Chevron, C.I.1111
Br.3139	Betzes, C.I.6398	Herta, C.I.8097	Prospect	Edda
Br.3962-4	Br.3902	Kindred, C.I.6969	Regal	Hannchen, C.I.531
G.B.76	Br.M45-680	Len.13	Ricardo, C.I.6306	M.C.3233
G.B.77	Br.M57-754	Leth.23	Rivale	Mianwali, C.I.3400
Heitpas 5, C.I.7124	Brant	Leth.27	Shaddock	Nepal, C.I.595
Husky	Byng	Leth.4362-3	Slovak	Ogalitsu, C.I.7152
Len.19	Carlsberg	M.C.6341	Svanhals	Olli
Lenta	Charlottetown 80	M.C.6940	Svansota	Parkland, C.I.10001
Lion, C.I.923	Chevalier, C.I.200	M.C.7940	Swan, C.I.10002	Tregal, C.I.6359
Opal B, C.I.6617	C.I.4974	McFadden, C.I.1443	Titan, C.I.7055	Valentine, C.I.7242
Rabat, C.I.4979	C.I.7252	Minerva	Trebi	Wong, C.I.6728
Sask.53156	C.I.7256	Modia, C.I.2483	U.M.584	2550 C
Traill, C.I.9538	Comfort, C.I.4578	Montcalm, C.I.7149	Velvet	3643 B
Vantage, C.I.7324	Duplex, C.I.2433	Moore, C.I.7251	Velvon 11, C.I.7088	
Vantmore, C.I.9555	Fort	Morocco, C.I.3902-1	Warrior	
Vaughn, C.I.1367	Fox, C.I.9190	Nord	Weibull's, 4468	
5025-8-2	Freja	O.A.C.21	Wis. H42, C.I.7123	
	Gatami, C.I.2276	Peatland	York	
	G.B.60	Piroline, C.I.9558	3092 D	

* C.I. refers to accession number of the Cereal Section, United States Department of Agriculture

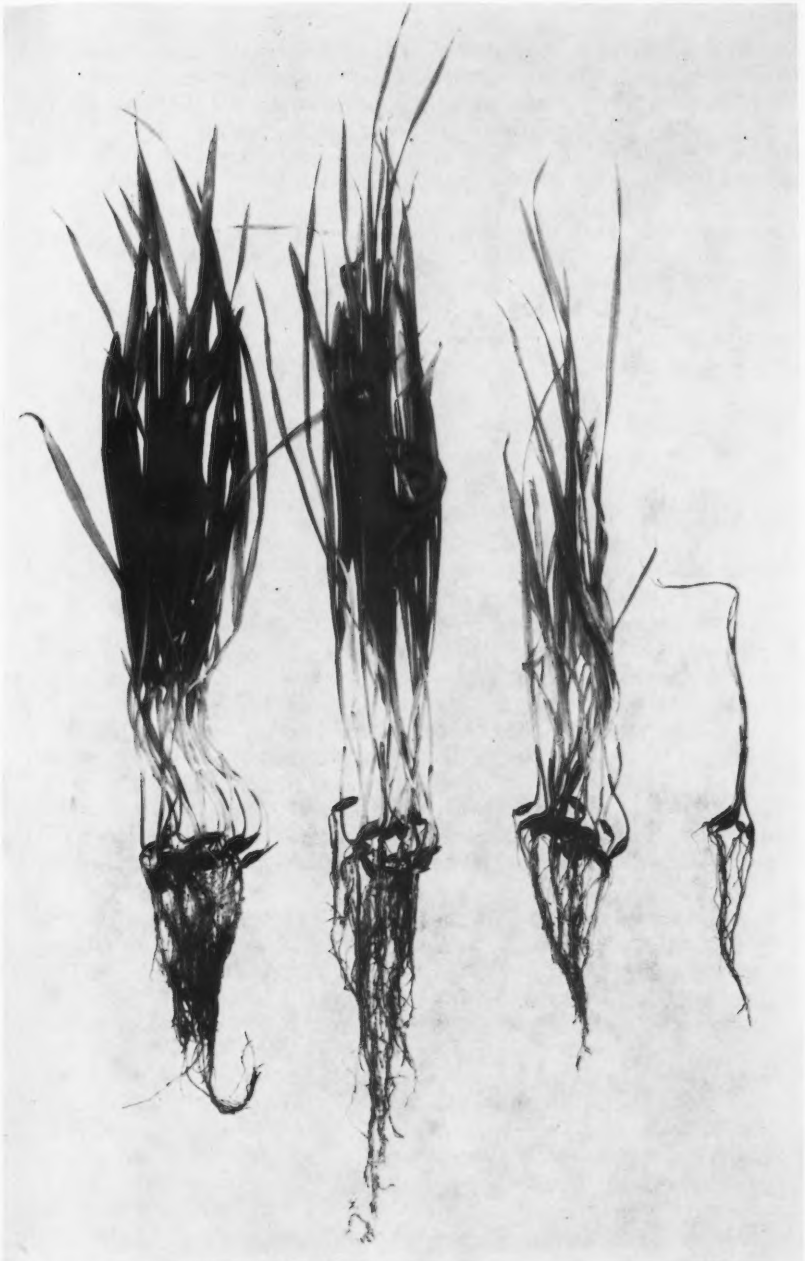

FIGURE 2. Seedlings of two resistant selections compared with resistant and susceptible check varieties. *Left to right*: two 4805 selections, Br. 3962-4 and Olli.

At the end of the growing period the seedlings were removed from the plant bands and washed free of soil or sand and inoculum. Several criteria were used to assess the reaction of the plants to root rot and seedling blight. In the early tests, the mean height of the plants (from kernel to leaf tip) in each replicate was recorded. The seedlings were also classified for the degree of coleoptile discoloration and lesioning and for the amount of blighting and stunting following the method used by Anwar (1). A disease index was then calculated from these data. In later experiments, each replicate of each variety or hybrid line under test was rated visually, after removal from the planting mixture, for resistance to root rot and seedling blight under the following classes: 1. *excellent;* 2. *very good;* 3. *good;* 4. *fair;* 5. *poor;* 6. *bad.* Photographs were taken of those tests which gave a good differential reaction and these were then used to standardize readings on subsequent tests.

RESULTS AND DISCUSSION

Methods of Inoculation for Testing Barley to Root Rot and Seedling Blight

According to Wood *et al.* (11), *H. sativum* comprises many parasitic races that differ in their ability to infect varieties of *Hordeum vulgare* and of other genera. In the present study, seven isolates from widely separated areas were compared using four varieties that differed in their reaction to the fungus, to determine if any differences existed in pathogenicity between isolates or in virulence between and within isolates. The Ottawa isolate was the most virulent one tested when the disease index and decrease in height were considered (Figure 1). The Macdonald College isolate (Quebec isolate of Figure 1), used by Ludwig *et al.* (8) and the Nappan isolate were also highly virulent but slightly less so than the Ottawa isolate. The other isolates were less virulent than the Nappan and Quebec isolates (Figure 1). However, there was no evidence of a differential response of these isolates to the test varieties. Therefore, in all subsequent varietal testing work at the Cereal Crops Division the most virulent (Ottawa) isolate was used.

A number of soil infestation levels were compared to determine the optimum level for varietal differentiation. These were 0, 1, 3, 5, 10, 20 and 30 per cent inoculum by weight. The lower levels of soil infestation (0, 1, 3, 5 per cent) did not give sufficient differentiation for varietal tests. Severe disease symptoms were produced at the higher concentrations (20 and 30 per cent) with a corresponding reduction in plant height. Soil infestation levels of 10 and 20 per cent were compared in a number of tests. The best degree of varietal differentiation was afforded by adding the inoculum to sandy soil at the 20 per cent level or to sterilized white silica sand at the 10 per cent level. Sterilized white silica sand gave more uniform results with the least expenditure of inoculum. An example of varietal differences in reaction is illustrated in Figure 2.

Micro-organisms other than *H. sativum*, carried on barley seed, may reduce seedling infection because of the poor competitive ability of this fungus. No differences were observed in a series of experiments, com-

paring surface sterilized and non-surface sterilized seed. However, surface sterilized seed was used throughout the tests to eliminate the possible effects of other organisms.

Sterilized and non-sterilized soil were compared to determine the optimum conditions for varietal testing. The results indicated that the micro-flora of non-sterilized soil had a depressing effect upon the severity of disease development. Therefore, sterilized soil or white silica sand were used in the variety and hybrid line testing.

Differences were observed in the host reaction to *H. sativum* when tests grown in compacted soil were compared with tests grown in loose soil. Differences in varietal reaction could readily be demonstrated in compacted soil but were much less evident in loose soil. A special tamper was constructed to obtain maximum contact of seed and inoculum and was used in all testing work.

Reaction of Varieties and Selections to Root Rot and Seedling Blight

A total of 600 varieties and selections were tested for reaction to the root rot and seedling blight phase of the disease caused by *H. sativum* (Ottawa isolate) during the course of this study. Ninety-seven of these were named varieties and more advanced selections. Five hundred and three were less advanced selections from 16 different hybrid populations.

TABLE 2. — DISTRIBUTION OF SEEDLING REACTIONS OF BARLEY SELECTIONS WHEN GROWN IN A MEDIUM INFESTED WITH THE OTTAWA ISOLATE OF *H. sativum*

Hybrid number	Parentage	Number of lines			
		Resistant	Intermediate	Susceptible	Total
4666	Vantage (R)* x Rabat (R)	0	10	0	10
4805	Anoidium (R) x Rabat (R)	25	190	36	251
4668	Byng (I) x Rabat (R)	0	1	0	1
4806	Anoidium (R) x Fort (I)	1	59	48	108
4811	Anoidium (R) x Fort[2] (I)	0	7	2	9
5026	Anoidium (R) x Montcalm[2] (I)	0	43	16	59
4682	Montcalm (I) x Fort (I)	0	20	9	29
4808	Velvon 11 (I) x Montcalm (I)	0	0	1	1
4646	Rabat (R) x Olli (S)	0	1	0	1
4684	Montcalm (I) x 3643 B ** (S)	0	1	0	1
4685	3643B (S) x Fort (I)	0	4	0	4
4635	S.C.46-235 (U) x Br.3962-4*** (R)	3	0	0	3
4636	Velvon 51 (U) x Br.3962-4 (R)	0	1	0	1
4637	Velvon 68 (U) x Br.3962-4 (R)	1	0	1	2
5233	5026 (U) x Montcalm (I)	2	0	0	2
5305	4805 (U) x Ogalitsu (S)	0	7	14	21

* R = resistant; I = intermediate; S = susceptible; U = unknown
** 3643B = Velvet x Olli 2 x Peatland 3 x Duplex
*** Br.3962-4 = Mensury x Newal 2 x Peatland 3 x Plush

Each variety and the majority of selections were tested several times and in no case was any entry tested less than twice. The variety and more advanced selection reactions are given in Table 1. Table 2 summarizes the reactions of the material from the various hybrid populations.

Nineteen entries in Table 1 were classified as resistant. Fifteen entries were susceptible. The remaining entries had an intermediate reaction. Anoidium, Br.3962-4 (Newal X Peatland 2X Plush 3X Mensury), Lenta and Opal B were the most resistant varieties and Olli was one of the most susceptible. A number of those entries with an intermediate reaction may actually be heterogeneous with respect to resistance because they were not selected for resistance to *H. sativum* during their development. Br.3962-4 was used as the resistant check in all experiments and varietal tests. (In recent experiments, Lenta and Opal B have been added as resistant checks). Olli was used as the susceptible check.

A total of 503 selections from 16 hybrid populations (Table 2) was evaluated for reaction to the Ottawa isolate of the fungus. All had been selected for characteristics other than root rot resistance. In many crosses the number of selections tested was too small to arrive at any conclusions about the inheritance of resistance. However, in crosses like 4805, 4806 and 5026 a large number of selections was tested. When both parents were resistant, a good proportion of resistant selections was recovered. When one parent was resistant and the other intermediate in disease reaction, the majority of selections were intermediate. When one parent was susceptible, few or no resistant selections were found.

The varieties tested in this study exhibited no differential response to the several isolates of *H. sativum* used. This is interpreted to indicate the absence of physiological races of the organism. Possibly a better understanding of the pathogenicity of *H. sativum* and the use of a differential series with a greater reaction range might distinguish races. However, the available information indicates that good resistance to root rot and seedling blight incited by *H. sativum* can be detected by the use of the present technique and can be used profitably in a barley-breeding program.

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CONTROL OF ROOT MAGGOTS ATTACKING CRUCIFEROUS CROPS, MAINLY RUTABAGAS, GROWN IN RIDGES[1]

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ABSTRACT

Experiments in 1958 and 1959 with pre-planting soil treatments of insecticides, applied in a 5-inch band 1½ inches below the seed in ridges of soil, showed that heptachlor, aldrin and chlordane, each at 5 pounds toxicant per acre, gave 70 to 90 per cent reduction in root maggot damage in cruciferous crops in Prince Edward Island. Each of these insecticides gave better control of the second than of the first generation of root maggots. Nematocide 18,133, at 5 and at 10 pounds per acre gave complete control throughout the growing season and also gave the highest yield. Thimet at 5 and at 10 pounds gave better control than the chlorinated hydrocarbons; parathion and Guthion, at the same rates, gave better control of the first generation but not as effective control thereafter. Korlan at 6 pounds gave fair control of the first generation but was ineffective thereafter. Ethion, Trithion, Di-syston and Diazinon, each at 5 and at 10 pounds, and Co-Ral and Dylox at 5 pounds were ineffective in controlling root maggot injury. Diazinon was highly phytotoxic; none of the other insecticides showed any phytotoxicity.

INTRODUCTION

Although infestations of root maggots attacking cruciferous crops, onions, and carrots were at first readily controlled with soil treatments of certain chlorinated hydrocarbon insecticides, resistant strains of the onion maggot, *Hylemya antiqua* Meig. soon developed and materials such as heptachlor and aldrin are no longer effective in controlling this pest in many areas of Canada and the United States [literature reported and reviewed by McClanahan *et al.* (3)]. Adequate control of the cabbage maggot, *H. brassicae* (Bouché), the major pest of cruciferous crops in many areas of Canada, has been obtained with heptachlor and aldrin (1, 2, 6), but this pest may also develop resistance to these insecticides. This is a report on experiments with various chlorinated hydrocarbon and organic phosphate insecticides against the cabbage maggot attacking cruciferous crops grown in ridges of soil in Prince Edward Island in 1958 and 1959.

METHODS AND MATERIALS

The insecticides and rates of application tested in plot experiments are given in Tables 1, 2 and 3. In each of the experiments there were four randomized blocks, each block having the treatments as shown in the tables, and three untreated plots. Each plot consisted of two rows, each 60 feet long. The rows were 2½ feet apart, the plots 3 feet apart, and the blocks 20 feet apart. The plots were arranged along one side of the test field, in an area adjoining a field known to have had a heavy infestation of root maggots the previous season. This ensured a severe infestation in the test plots, and minimized the variations in exposure to infestation between and within blocks.

In whole-field tests at Pownal, comprising 3 to 5 acres of crop, heptachlor and parathion were applied to the whole row. Making alternate

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TABLE 1. — NUMBERS OF LARVAL TUNNELS PER 100 RUTABAGAS, DAMAGE INDEXES AND PERCENTAGE MARKETABLE ROOTS AFTER TREATMENTS WITH VARIOUS INSECTICIDES AT TYRON, PRINCE EDWARD ISLAND, 1958[1]

Insecticide[2]	Toxicant lb./acre	Larval tunnels[3]	Damage index[4]	Marketable roots (per cent)
Untreated	—	1069	94	7
Aldrin	8	52 (95)	7	94
Heptachlor	5	56 (95)	4	94
Parathion	3	69 (94)	4	93
Heptachlor	3	82 (94)	12	91
Heptachlor*	5	137 (88)	16	87
Aldrin	5	182 (83)	32	71
Parathion	3	259 (76)	30	74
Thimet	4	329 (69)	29	70
Thimet	7	400 (63)	40	61
Disyston	4	788 (26)	78	26
Guthion	5	876 (18)	70	33
Disyston	2	1013 (5)	88	10
Ethion	5	1032 (3)	90	9
Ethion	3	1043 (3)	92	9
Malathion	5	1080 (−1)	95	4
Malathion	3	1162 (−9)	97	4
Difference required for significance at 5% level		170		
at 1% level		227		

* Wettable powder formulation applied with a hand applicator
[1] Except for one treatment of heptachlor, all of the insecticides (5 or 10 per cent granular) were applied in a 5-inch band 1½ inches below the seed in a ridged drill with a 2-row subsurface insecticide applicator
[2], [3], [4]. See corresponding footnotes in Table 2

applications of the two insecticides, 10 rows were treated and two left untreated. The tests were located on different fields of the same farm each year, each experiment being situated as close as practical (never more than 100 yards) to the previous season's rutabaga crop.

Except for the hand application indicated in Table 2, the insecticides were applied in a 4- to 5-inch band about 1½ inches below the seed in a ridged row with the subsurface insecticide applicator described by Read (5). The machine was altered slightly in that the space-planting seeder, with some alteration, was mounted on the machine directly behind the insecticide hoppers, thus providing precise placement of the seed in respect to the location of the band of insecticide and making the combined machines much easier to operate in the field.

The plot experiments were conducted in sandy loam soil areas where the first generation of root maggots occurs mainly in late June and July and the second generation in September and October (4). To ensure the heaviest possible infestations throughout the season, the crops were planted during the first 2 weeks of June. In the whole-field tests, conducted in a clay loam soil where the first generation attack does not begin until late in July, the plots were planted during the last week of June.

The Laurentian variety of rutabagas was used in all of the tests. Standard varieties of early cabbage, early cauliflower, broccoli and brussels sprouts (planted in the field as seed) were treated with heptachlor, Experimental Nematocide 18,133, parathion, and Thimet in one experiment in 1959.

TABLE 2. — NUMBERS OF LARVAL TUNNELS PER 100 RUTABAGAS, RECORDED FOR BOTH EARLY AND LATE ATTACKS, AND DAMAGE INDEXES, AFTER TREATMENTS WITH VARIOUS INSECTICIDES AT DIFFERENT RATES, COVEHEAD, PRINCE EDWARD ISLAND, 1959[1]

Insecticide[2]	Toxicant lb./acre	Larval tunnels[3]			Damage index[4]
		Early	Late	Total	
Untreated	—	487	568	1054	96
18,133	5	0	0	0	0
18,133	10	0	0	0	0
Thimet	6	3 (99)	25 (96)	28 (99)	3
Thimet	12	4 (99)	16 (97)	20 (99)	2
Parathion	10	23 (95)	48 (91)	71 (93)	11
Guthion	10	52 (89)	141 (75)	194 (82)	17
Parathion	5	69 (86)	158 (70)	227 (79)	28
Chlordane	10	120 (75)	93 (84)	213 (78)	30
Heptachlor	5	199 (52)	103 (82)	302 (72)	21
Guthion	5	132 (73)	207 (64)	339 (68)	34
Parathion	5	169 (69)	176 (64)	345 (68)	45
Chlordane	5	188 (62)	180 (68)	368 (65)	46
Aldrin	6	246 (51)	221 (62)	467 (58)	55
Korlan	6	169 (65)	376 (34)	545 (48)	63
Diazinon	5	346 (29)	341 (40)	684 (35)	86
Trithion	10	360 (26)	524 (8)	884 (16)	89
Dylox	5	358 (27)	564 (1)	923 (13)	92
Trithion	5	373 (23)	551 (3)	924 (12)	91
Ethion	10	446 (8)	501 (12)	948 (10)	89
Co-Ral	5	393 (19)	574 (−1)	967 (8)	92
Ethion	5	458 (6)	578 (−1)	1036 (2)	96
Difference required for significance at 1% level		130	122	190	
at 5% level		97	92	143	

[1]Early (first generation) root maggot attacks occurred during late June, July, and early August; late (second and third generation) attacks occurred mainly during September and October

[2]Granulated formulations (5 or 10 per cent) of all of the insecticides excepting Korlan and Co-Ral were used.

Aldrin, 1,2,3,4,10,10-hexachloro-1,4,4a,5,8,8a-hexahydro-1,4-*endo*, *exo*-5,8-dimethanonaphthalene (Niagara Brand Spray Co. Ltd., Burlington, Ont.)

Chlordane, 1,2,4,5,6,7,8,8-octachloro-3a,4,7,7a-tetrahydro-4,7-methanoindan (Velsicol Chemical Corp., Chicago, Ill.)

Co-Ral, (25% wettable powder) *0,0*-diethyl *0*-(3-chloro-4-methyl-7-coumarinyl) phosphorothioate (Chemagro Chem. Corp., Kansas City, Missouri)

Diazinon, *0,0*-diethyl *0*-(2-isopropyl-6-methyl-4-pyrimidinyl) phosphorothioate (Chipman Chemicals Ltd., Hamilton, Ont.)

Di-syston, *0,0*-diethyl *S*-2-(ethylthio)ethyl phosphorodithioate (Chemagro Chem. Corp., Kansas City, Missouri)

Dylox. dimethyl-(2,2,2-trichloro-1-hydroxyethyl) phosphonate (Chemagro Chem. Corp., Kansas City, Missouri)

Ethion, *0,0,0*^{1},*0*^{1}-tetraethyl *S,S*^{1}-methylene bisphosphoro-dithioate (Niagara Brand Spray Co. Ltd., Burlington, Ont.)

Experimental Nematocide 18,133, *0,0*-diethyl *0*-2-pyrazinyl phosphorothioate (American Cyanamid Co., Linden, New Jersey)

Guthion, *0,0*-dimethyl *S*-(4-oxo-3H-1,2,3-benzotriazine-3-methyl) phosphorodithioate (Chemagro Chem. Corp., Kansas City, Missouri)

Heptachlor, 1(or 3a), 4,5,6,7,8,8-heptachloro-3a,4,7,7a-tetrahydro-4,7-methanoindene (Velsicol Chemical Corp., Chicago, Ill.)

Korlan (25% wettable powder), *0,0*-dimethyl *0*-(2,4,5-trichlorophenyl) phosphorothioate (Dow Chemical of Canada Ltd., Toronto, Ont.)

Malathion, *0,0*-dimethyl phosphorodithioate, *S*-ester with diethyl mercaptosuccinate (American Cyanamid Co., New York, N.Y.)

Parathion, *0,0*-diethyl *0*-*p*-nitrophenyl phosphorothionate (Hopkins Agricultural Chemical Co., Randolph, Wisconsin)

Thimet, *0,0*-diethyl *S*-(ethylthio)methyl phosphorodithioate (American Cyanamid Co., Chicago, Ill.)

Trithion, *0,0*-diethyl *S*(*p*-chlorophenylthio)methyl phosphorodithioate (Stauffer Chemical Co., New York, N.Y.)

[3]Figures in parentheses are percentages of control

[4]*After* King and Forbes (J. Econ. Entomol. 47: 607-615. 1954)

Records of injury in all of the experiments were taken during the last 2 weeks of October and the first week of November. Except for the treatments of Experimental Nematocide 18,133, where 125 plants per plot were examined, 50 plants taken at random in each plot were examined for root maggot injury. Appraisal of control for rutabagas was based on the number of larval scars or tunnels resulting from first-generation attacks and the number of tunnels from late- or second-generation attacks.

The two main attacks were easily distinguishable because the first occurred when the plants were growing rapidly and hence resulted in distortion of the plant or in wide scars, whereas the second attack, occurring when the plants were nearly full grown, resulted in narrow tunnels circumscribing the roots and slight internal tunnelling if the soil was dry. If there were more than about six tunnels per plant resulting either from early or late injury, the number was estimated. It was, of course, not possible to count the exact number of tunnels in a severely infested plant and, although more than 60 larvae have been found in one plant, the estimated number was limited to 12 scars or tunnels from early injury and 12 tunnels from late injury. Thus the error in estimate was in favour of the recorder. The tunnel-count method provided an accurate estimate of the effectiveness of an insecticide in controlling infestations of root maggots throughout the growing season. The damage index method by King and Forbes (2) was used to categorize the amount of injury to all of the cruciferous crops tested. Yield records were taken on the cabbage and cauliflower.

All results were tested for significance by an analysis of variance. Tests for residues of heptachlor, aldrin, parathion, and Thimet were made on rutabaga samples collected at time of harvest from experiments conducted in 1958.

RESULTS

Tables 1, 2, and 3 show the infestation and injury after treatments with various chlorinated hydrocarbon and organic phosphate insecticides. The percentage of marketable roots in a replicate receiving a certain treatment can be estimated approximately by subtracting the appropriate damage index from 100. In estimating the commercial value of the treatments, any measure above 70 per cent was considered very good control.

In the 1958 tests (Table 1) aldrin, heptachlor, and parathion gave good control of root injury. Thimet gave about 65 per cent control but the results were questionable in that the 4-pound application appeared to give better results than the 7-pound application; the insecticide contained a vermiculite carrier which made the material difficult to distribute with the machine (in the 1959 tests an attaclay carrier was used). Guthion gave good control of the first generation of root maggots but was ineffective later in the season; the chemical was considered to be of poor quality and a new supply was tested in 1959. Di-syston, malathion and ethion gave little or no protection against cabbage maggot injury. Ethion, however, was again tested in 1959 at higher rates, since it had given good control of the onion maggot (3).

In the 1959 plot experiments aldrin, chlordane, parathion, Guthion, heptachlor, Thimet and Experimental Nematocide 18,133, gave fair to excellent control of the cabbage maggot. Korlan gave fair control of early attacks but only slight control thereafter. Trithion, Dylox, Co-Ral, ethion and Diazinon gave little or no control. Diazinon was highly phytotoxic; at the 5-pound rate more than 80 per cent of the plants were killed, those surviving having massive hairy root systems; at 10 pounds almost all of the plants were killed. None of the other insecticides tested showed any signs of phytotoxicity.

The chlorinated hydrocarbon insecticides gave better control of the late- or second-generation cabbage maggot attacks than of earlier attacks, while all of the organic phosphate materials gave better control of the early attacks (Table 2). At York, parathion gave better control of early attacks than heptachlor but only slight to moderate control of late attacks, whereas heptachlor gave slightly better control of the later than of the early attacks (Table 3). This phenomenon was equally apparent in two other control experiments planted in June, 1959, one at Pownal, Prince Edward Island, and one at Nappan, Nova Scotia.

The results at Covehead in 1959 (Table 2) indicated that heptachlor was not so effective against the cabbage maggot as in previous years (6). However, almost complete control was obtained in fields where this insecticide was used commercially. At Pownal, where whole-field tests were conducted on the same farm for 5 consecutive years, the numbers of larval tunnels per 100 rutabagas in untreated- and insecticide-treated rows (figures in parentheses are percentages control) were as follows:

Treatment	1955	1956	1957	1958	1959
Untreated	398	318	640	459	1064
Heptachlor, 5 lb.	37 (91)	1 (99)	80 (89)	0 (100)	68 (94)
Parathion, 5 lb.	—	—	—	4 (99)	94 (91)

Heptachlor, parathion, Experimental Nematocide 18,133, and Thimet gave the same degree of protection against injury by the cabbage maggot to cabbage, cauliflower, broccoli and brussels sprouts grown directly from seed in the field as to rutabagas; the damage index figures for the treatments at 5 pounds toxicant per acre to these crops were the same as those shown for rutabagas in Table 2. In the untreated check plots most of the cauliflower and many of the cabbage plants were destroyed by the maggots. Less than 1 per cent of the broccoli and brussels sprouts were actually killed, although the yields were significantly reduced.

For transplants of the stem brassicas, the same high degree of control was obtained with 2 pounds of insecticide per acre placed around the stems of the plants about 3 days after transplanting in the field and covering the insecticide lightly with soil. Since the tests with rutabagas showed the effectiveness of the insecticides against the cabbage maggot, the data from the tests with the stem brassicas are not included in this report.

There was no significant difference in the degree of control between replicates in any of the experiments.

TABLE 3. — NUMBERS OF ROOT MAGGOT TUNNELS PER 100 RUTABAGAS, AND DAMAGE INDEXES FOLLOWING TREATMENTS OF HEPTACHLOR AND PARATHION AT TWO RATES IN AN AREA WHERE DRY WEATHER IN JULY NOTICEABLY RETARDED DEVELOPMENT OF THE PLANTS, YORK, PRINCE EDWARD ISLAND, 1959

Insecticide[2]	Toxicant lb./acre	Larval tunnels[3]			Damage index[4]
		Early	Late	Total	
Untreated	—	690	628	1318	98
Heptachlor	10	70 (90)	22 (96)	92 (93)	4
Heptachlor	5	125 (82)	46 (93)	171 (87)	19
Parathion	10	20 (97)	327 (48)	347 (74)	22
Parathion	5	90 (87)	587 (7)	677 (49)	48
Difference necessary for significance at 1% level		123	190	279	
at 5% level		88	136	199	

[2, 3, 4] See corresponding footnotes for Table 2

Residue analyses on samples of the rutabagas treated with heptachlor, aldrin, parathion, and Thimet showed that the amounts of these insecticides absorbed by the plants were well below the tolerance limits established for Canada. The residues detected in samples collected at time of harvest were as follows: heptachlor from tests conducted at four localities in Prince Edward Island, Nova Scotia and New Brunswick, none; aldrin, none (dieldrin, none); parathion, 0.1 p.p.m.; Thimet, none. Analyses were expressed in parts per million on a fresh weight basis. Tests made for residues of heptachlor on the peel of rutabagas showed 0 to 0.004 p.p.m. retained on or in the surface of the roots. Residues of heptachlor epoxide on rutabagas from tests conducted in 1959 were as follows: peel, 0 to 0.02 p.p.m.; pulp, 0 to 0.015 p.p.m. No method had been determined, as of January 1960, for detecting residues of E.N. 18,133 in rutabagas.

DISCUSSION

The applications of chlorinated hydrocarbon insecticides tested in Prince Edward Island gave effective protection against cabbage maggot injury throughout the whole growing season, but no tests were conducted to determine why heptachlor and aldrin were more effective 4 or 5 months after application than during the first 3 months (Table 2). Results of the residue tests for heptachlor indicate that there was a breakdown of the original compound to its epoxide and the epoxide may have been more toxic to the larvae. However, the increase in effectiveness could simply have been due to the fact that, as the plants developed, the bulbous portion of the roots expanded outward into the band of insecticide and provided a higher concentration immediately surrounding the roots. During the early season, the newly-hatched maggots may have been able to move down by the stems of the plants and feed on the roots below, and without coming in contact with a lethal dosage of, the poison.

Organic phosphate insecticides generally have a much shorter period of residual effectiveness than the chlorinated hydrocarbons, and the gradual loss in effectiveness of the materials for which data are given in Table 2 was probably a normal breakdown in the chemical. At York and Nappan, the rate at which breakdown occurred was increased by warm, dry soil conditions and parathion gave effective protection against root maggot attacks for only about 2 months. However, in the other experiments conducted during 1958 and 1959, parathion gave excellent root maggot control for the whole growing season and almost complete control was obtained with Thimet and Experimental Nematocide 18,133. It was indicated that the period of residual effectiveness of such organophosphorous compounds was markedly prolonged by placing the treatments in a narrow band in a shallow ridge of soil and covering the treatments with about an inch of soil. At the time of planting, 2 inches of soil were placed over the insecticide, but about half of this was removed during cultivating and thinning of the plants.

A comparison of the results of tests on cabbage maggot control reported herein with results obtained against the strains of the onion maggot resistant to aldrin, etc., in Ontario, British Columbia and elsewhere (3) shows that (a) ethion, Trithion, Co-Ral, Dylox and Diazinon have given protection against the resistant strains of the onion maggot but, with the methods of application used in Prince Edward Island, these insecticides were ineffective against the cabbage maggot – even when applied at three or more times the rates found to be effective against the onion maggot; (b) Experimental Nematocide 18,133, Thimet, and parathion gave better control of the cabbage maggot than of the onion maggot. It is, therefore, indicated that there are probably notable physiological differences between the two species in that one is immune to certain organic phosphate insecticides while the other is quite susceptible.

The first evidence of resistance of the cabbage maggot to the chlorinated hydrocarbons, heptachlor and aldrin, occurred in the northwestern United States and in British Columbia in 1959, and researchers in these and other regions must thoroughly investigate the organophosphorus compounds and should not anticipate that the materials used to control strains of the onion maggot that are resistant to heptachlor and aldrin will also control the cabbage maggot.

The machine used to make the seeding ridges, apply the insecticide treatment at the required depth in the soil, and sow rutabaga seed was found equally efficient for other cruciferous crops grown from seed planted directly in the field. However, for protecting transplants against root maggot injury it is considered preferable to use the hand-applicator method described by Read (6); because of the 15- to 24-inch spacing of the plants, much less insecticide is required to obtain protection.

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POTASSIUM IN PLANT METABOLISM.

III. SOME CARBOHYDRATE CHANGES IN THE WHEAT SEEDLING ASSOCIATED WITH VARYING RATES OF POTASSIUM SUPPLY[1]

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ABSTRACT

Wheat seedlings grown in sand culture responded to decreased potassium application with increases in reducing sugar content and in invertase activity. The pH of the seedling press juice and the K_M of the invertase preparations both diminished as the potassium supply to the seedlings was decreased. Potassium in the enzyme digest had no effect on the level of activity of the individual invertase preparations.

INTRODUCTION

The relationship of potassium to carbohydrate metabolism in plants has been the subject of many investigations. The problem can be studied in at least two ways, first by measuring the levels and fluctuation rates of carbohydrate metabolites under varying conditions of potassium nutrition and, second, by determining the activity levels of the relevant enzymes. Quantitative studies have shown that certain general relationships are consistently found in many species of plants – potassium deficiency usually results in decreased concentrations of the more complex carbohydrate molecules and corresponding higher concentrations of the simpler saccharides (6, 7). Whether this is due to increased hydrolytic activity or to inhibition of the mechanism of synthesis is not very clear although it has been reported that potassium deficiency stimulates the activity of certain hydrolytic carbohydrate enzymes (1, 3, 4, 6). A previous paper in this series (14) reported that the percentage of starch in potato leaves was directly proportional to the amount of potassium supplied to the plants. The present study deals with the wheat seedling which does not store starch as a primary reserve material and is representative of a different type of carbohydrate economy. The levels of sucrose and reducing sugars have been measured in seedlings grown under a wide range of potassium treatments and the activity of one of the relevant enzymes, invertase, has been studied under the same conditions. The aim of this series of investigations is to determine direct or indirect effects of potassium in physiological processes.

MATERIALS AND METHODS

Fourteen-day wheat (*Triticum aestivum* L.) seedlings of the variety Acadia, were grown in sand culture either in a growth chamber in the manner described previously (13) or in a greenhouse. The levels of potassium supplied to the plants in the nutrient solutions varied for different experiments as indicated in the tables of data. In each instance, nitrogen was maintained at a constant level by supplying $NaNO_3$ where KNO_3 was reduced. In some experiments, the complete aerial portion of the seedling was used for analysis; in others, the plants were separated into three parts – *first leaf*, *second leaf* and *stem.*

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TABLE 1. — EFFECT OF VARYING RATES OF POTASSIUM APPLICATION UPON THE REDUCING SUGAR AND SUCROSE CONTENT OF 14-DAY WHEAT SEEDLINGS GROWN IN SAND CULTURE (EXPRESSED AS PER CENT OF FRESH WEIGHT)

K treatment m. eq./l.	Reducing sugars					Sucrose				
	Chamber		Greenhouse			Chamber		Greenhouse		
	Whole plant	Whole plant	First leaf	Second leaf	Stem	Whole plant	Whole plant	First leaf	Second leaf	Stem
0	.45	.50	.92	1.59	1.73	.19	.35	.85	1.25	.50
0.25	.44	.42	.97	1.47	1.19	.12	.31	.90	.78	.47
0.50	.35	.35	1.17	1.24	1.38	.18	.26	1.15	.86	.50
1.0	.29	.39	1.20	1.39	1.23	.17	.30	1.11	1.06	.27
2.5	.34	.46	1.46	1.36	1.38	.26	.35	1.04	.96	.34
5.0	.28	.20	1.00	1.15	1.19	.26	.17	1.16	1.10	.29
10.0	.18	.15	.65	.83	1.07	.24	.20	.50	.69	.49
Correlation coefficient r_{yi}	−.86	−.81		−.92	−.62	+.64	−.41		−.20	+.07

Value of r at the 5% level of significance = .754

Reducing sugars and sucrose were determined by methods described previously (12). Measurements of invertase activity were made on undiluted press juice obtained from freshly harvested plant tissue and cleared by centrifuging at 20,000 x *g* for 15 minutes. The enzyme digests consisted of 4 ml. phosphate-citrate buffer at pH 4.5, 5 ml. 2 per cent sucrose, 1 ml. cleared plant juice. A digestion time of 15 minutes was adopted. Data for invertase are expressed in terms of the international unit of enzyme activity (5) defined as the amount of activity required to utilize 1 micromole of substrate per minute under specified conditions of pH and temperature. For purposes of comparison the activity thus calculated is referred to the tissue of origin by expressing it as units of activity per gram of fresh tissue.

RESULTS

For the purpose of measuring the effect of potassium on reducing sugars and sucrose in the wheat seedling, plants were grown with seven levels of potassium in the nutrient solution ranging from 0 to 10 milliequivalents per litre. Three tests were made, two with chamber-grown plants in which the whole aerial portion of the plant was analysed as one sample, and a third test in which separate analyses of first leaf, second leaf and stem were made on seedlings grown in a greenhouse. The data presented in Table 1 show that there is a significant relationship between rate of potassium application and level of reducing sugars in whole plants and in the second leaf of dissected plants. The relationship does not hold for the first leaf for the following reasons. The effects of potassium deficiency are most severe in the first leaf (13). After 14 days the tips have become necrotic, metabolism is disorganized, and relationships such as the one described above are largely obscured. The same applies in part to the stem tissue while the second leaf remains green and vigorous, although with a slightly altered metabolism. The data for sucrose in Table 1 show that there is no direct relationship between rate of potassium application and sucrose content in whole plants or in any part of the plant. Although greenhouse plants were used for a part of this experiment and higher levels of sugars were obtained, the same potassium-reducing sugar relationship may be observed in Table 1.

The first phase of the invertase study was made with samples of expressed juice from seedlings grown with seven levels of potassium in the nutrient solution as above. The results as shown in Figure 1 indicate that invertase activity increased with decreasing rate of potassium application but the effect was more pronounced with the lower levels of potassium concentration. Levels of enzyme activity are affected by pH, temperature, substrate concentration, co-factors and inhibitors. In seeking an explanation of the rise in invertase activity that accompanies potassium deficiency several of these factors were studied. The pH levels of the wheat leaf press juices mentioned above were measured and the values have been plotted in Figure 1 along with the enzyme activities. The pH of the juice increased slightly with increasing potassium but this would not account for the enzyme effect since all the activity tests were measured in buffered digests at pH 4.5 which is the pH of optimum activity for the enzyme.

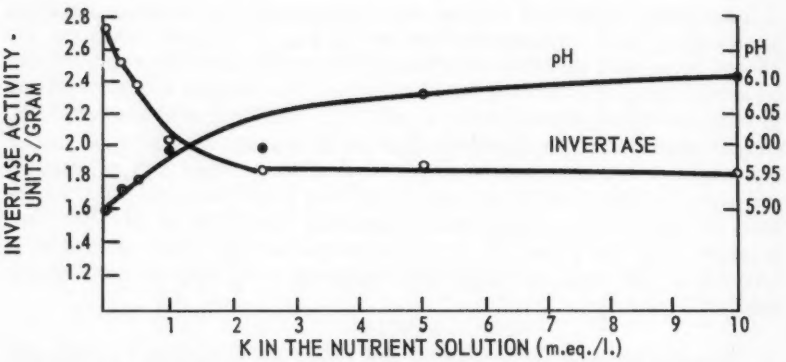

FIGURE 1. Effect of rates of potassium application upon the pH and invertase activity of 14-day wheat seedlings grown in sand culture.

Furthermore, the magnitude of activity differences is too great to be explained by the small range of pH change, i.e. 5.90 – 6.11.

An experiment was carried out to determine whether potassium ions in the digest have any effect on invertase activity. The effects of some other ions were tested at the same time. Two samples of press juice were prepared, one from seedlings supplied with an adequate quantity of potassium for normal growth and the other from seedlings supplied with no potassium. These are called + K and – K. Portions of each juice were dialysed. Salts in various concentrations were added to enzyme digests and the resulting activities measured. The results are shown in Table 2. Dialysis caused some loss of activity in all juices. It effectively removed all the potassium but this did not result in any rise in activity which would be expected if the reduction of potassium ion concentration were directly responsible for the stimulation of activity found in a – K juice. The

TABLE 2. — EFFECT OF ADDED SALTS IN THE DIGEST UPON THE INVERTASE ACTIVITY OF WHEAT LEAF SEEDLING PRESS JUICE
(Expressed as units/g. fresh tissue)

Salt added (digest concentration)	+ K Seedlings		– K Seedlings	
	Undialysed	Dialysed	Undialysed	Dialysed
None	2.03	1.85	2.74	2.26
0.5M KNO_3	1.97			
0.3M KNO_3	2.04			
0.2M KNO_3	2.06			
0.1M KNO_3	1.86	1.63	2.53	2.17
0.05M KNO_3	1.95			
0.01M KNO_3	2.12	1.81	2.92	1.73
0.01M KCl	2.03	2.01	2.76	1.96
0.1M $MgCl_2$	.41	.08	.48	.35
0.01M $MgCl_2$	1.84	1.54	2.50	2.09
0.01M $MgSO_4$	1.79	1.56	2.53	1.74
0.01M $NaNO_3$	2.06	1.74	2.81	

TABLE 3. — VARIATIONS IN KM OF INVERTASE FROM 14-DAY WHEAT SEEDLINGS GROWN WITH DIFFERENT LEVELS OF POTASSIUM

K treatment m.eq/l.	KM molar sucrose
0	.0102
0.25	.0111
1.0	.0126
10.0	.0149

addition of KNO_3 in 0.1 M concentration caused a depression in the activity of all juices but the results of the addition of this salt in other concentrations were quite inconsistent. Salts of magnesium depressed invertase activity. $MgCl_2$ in 0.1 M concentration almost inactivated the enzyme. Sodium nitrate in the concentration used had no effect.

The Michaelis constant of invertase, i.e. the molar substrate concentration required to produce one-half the maximum reaction velocity, is known to vary with the source of the enzyme (11). For this reason a study of the Michaelis constant of wheat leaf invertase was carried out. Four sets of seedlings were grown in the greenhouse at four of the seven levels of potassium application used in two earlier experiments. The invertase K_M of the press juice from each of these samples was determined by standard procedure (11). The results given in Table 3 show quite clearly that the K_M increased in direct proportion to the amount of potassium supplied to seedlings.

DISCUSSION

The study of the influence of potassium upon certain aspects of carbohydrate metabolism is being carried out by making a systematic survey of the behaviour of various metabolites and some of the enzymes that control their synthesis or breakdown. For this purpose species of plants representing different carbohydrate systems have been grown under varying conditions of potassium nutrition. The principal finding in studies of this type by many investigators (6, 7, 14) has been that, under conditions of potassium deficiency, polysaccharide reserve substances are not accumulated to the same extent as under adequate potassium nutrition although they are never entirely absent.

Starch is the primary carbohydrate reserve material of the leaves of many dicotyledonous plants. It is not normally found in the leaves of monocotyledons although it is an important constituent of other organs. Fructosans of various structures have been isolated from the leaves of monocotyledons. Fructosans are found in the wheat plant but are present in substantial amounts as polysaccharide reserves only after the plant begins to mature. In the seedling they have not been considered reserve materials; this role seems to be taken by sucrose (9). The two situations are not strictly analogous; yet, because the accumulation of starch in potato leaves

was found to be a function of potassium concentration, a similar relationship between potassium and sucrose was sought in the wheat seedling. Experimental evidence shows that such a relationship does not exist. The reduction of polysaccharides in potassium-deficient dicotyledonous leaves is usually accompanied by an accumulation of hexose sugars. This suggests either a block in the synthesis or a stimulation of the hydrolysis of polysaccharides. In wheat leaves, although the net amount of sucrose was not diminished, reducing sugars accumulated with decreasing potassium. Further evidence for the influence of potassium here is the fact that invertase activity was shown to increase with decreasing potassium application to the plants. This could certainly account for the increase in reducing sugar content but it is unlikely that a significant increase in reducing sugars could occur from invertase action without a corresponding decrease in sucrose content. Therefore, since sucrose content changes so little, the effect of the greater invertase activity must be small in comparison with other possible means of bringing about an accumulation of reducing sugars such as inhibition of glycolysis or greater hydrolysis of oligosaccarides or polysaccharides.

Additional tests were made with wheat leaf invertase to ascertain the basis of its relation to potassium. The pH measurements of expressed juice have shown that, although there is a rise in pH with increasing potassium, the amount of change is so small that it would not account for the greatly increased enzyme activity at the lower pH levels.

The ion addition tests have ruled out the remote possibility of an essential ion effect with this invertase. The possibility of a natural inhibitor being present in normal tissue extracts but absent or reduced in potassium-deficient tissue extracts has not yet been investigated.

It has been pointed out that the K_M of wheat leaf invertase increases with increasing potassium application to the plants. This is an indication that some change has occurred in the enzyme itself rather than in the environmental conditions associated with its activity. A naturally occurring inhibitor would have the effect of inactiviating part of the enzyme at high potassium levels thus resulting in a greater substrate requirement and a higher K_M. Indeed Srinivasan and Quastel have shown that amino sugars inhibit the hydrolytic activities of yeast invertase (8) but more data are required for a complete explanation.

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THE INFLUENCE OF TEMPERATURE ON THE RATE OF ACID LOSS IN McINTOSH APPLES[1]

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ABSTRACT

Data are presented which suggest that the rate of loss of total titratable acids in the McIntosh apple varies exponentially with the storage temperature. This relationship may be expressed as $a = D.e^{ET}$ (D, E, are constants, T = storage temp. − 28.5 F., approx. freezing point). Certain published data on Bramley's Seedling apple are examined which tend to confirm and generalize the above finding.

INTRODUCTION

Acidity or tartness has long been regarded as a desirable attribute of apples. Acidity can be estimated by titration and is one of the few characteristics related to apple quality that can be easily and objectively measured. Because of this, the determination of total titratable acids is frequently relied on as a means of quality control for marketing both the fresh and the processed product. The investigator also relies on the use of this measurement in evaluating the effects of pre- and post-harvest treatment on the post-harvest physiology of apples.

The extensive investigations of Haynes (3) and Haynes and Archbold (4) in England have linked total titratable acids with storage life and the onset of disorders. Furthermore, these authors described the decline of acidity in terms of a locus of best fit which they suggested could be used to forecast storage life. This locus or function involved the computation of acid loss in terms of a seasonal constant for a particular temperature. This constant has been examined by Kidd *et al.* (5) who found that its value accelerated with higher storage temperatures.

The adherence of rates of acid loss to a descriptive mathematical relation meant that data could be more accurately assessed and presented in calculable and concisely organized form. The authors felt, however, that the storage forecasting aspect of the Haynes' relation could be enhanced if it were generalized in terms of temperature. For instance, in a generalized relation, titrations on representative samples held at high temperatures would be translatable into conditions at low temperature. And thus, through projection, the storage life of apple stocks held at normal or low temperature could be estimated months in advance.

An opportunity to study the temperature effect arose, as a side investigation, when non-replicated samples of juice were being prepared from apples held at various temperatures.

METHODS AND MATERIAL

Fruit was harvested in each one of 3 successive years, from two to four McIntosh trees of uniform size and vigour, and which bore normal crops. The fruit was identified as to tree and distributed in storage in equal quantities without grading.

[1]Contribution No. 50 from the Plant Research Institute, Canada Department of Agriculture, Ottawa, Ont.

TABLE 1.—TOTAL TITRATABLE ACIDS IN McINTOSH APPLES PICKED SEPT. 16, 1955, AND STORED AT INDICATED TEMPERATURES. SHOWN ALSO ARE THE RATES OF ACID LOSS, AND THE DEVIATIONS FROM THE LINES OF BEST FIT

	30°F.		32°F.		35°F.		40°F.		50°F.	
Days in storage	ln titr'n m.e. baryta per 100 ml. juice	Deviation expressed as ln	ln titr'n m.e. baryta per 100 ml. juice	Deviation expressed as ln	ln titr'n m.e. baryta per 100 ml. juice	Deviation expressed as ln	ln titr'n m.e. baryta per 100 ml. juice	Deviation expressed as ln	ln titr'n m.e. baryta per 100 ml. juice	Deviation expressed as ln
18	2.22	−.061	2.14	−.056	2.24	+.004	2.14	−.051	2.08	−.031
25									2.08	+.036
33	2.21	−.006	2.13	+.003	2.19	+.035	2.13	+.030	2.01	+.040
39									1.91	+.001
47	2.14	−.019	2.14	+.074	2.09	+.006	2.01	+.009	1.83	+.001
53					2.07	+.021			1.82	+.049
60	2.10	−.002	2.02	+.016	1.99	−.024	1.95	+.023	1.67	−.037
67							1.88	+.003	1.48	−.164
74	2.08	+.032	1.95	+.001	1.90	−.039	1.83	−.001	1.60	+.024
88	2.03	+.042	1.88	−.001	1.91	+.044	1.79	+.047	1.52	+.080
102	1.99	+.058	1.77	−.048	1.75	−.043	1.62	−.033		
116	1.88	+.008	1.76	+.003	1.62	−.103	1.51	−.055	1.39	+.217
130	1.81	−.003	1.70	+.011	1.68	+.037	1.50	+.027	1.27	+.237
151	1.73	−.001	1.60	−.003	1.60	+.062	1.43	+.089	1.12	+.287
207	1.45	−.049	1.31	−.043	1.30	+.056	1.03	+.048	0.82	+.523
Rate of acid loss:	"a" = 0.00414		"a" = 0.00449		"a" = 0.00526		"a" = 0.00638		"a" = 0.00961	

The storage temperatures were 30°, 32°, 35°, 40°, and 50°F. during the 1955-56 and the 1957-58 seasons. In 1956-57, 55°F. was substituted for the 50°F. temperature; otherwise temperatures were the same. Temperatures were controlled within ±0.5°F. and the relative humidity was controlled in excess of 90 per cent. Weight loss of the apples was 3 per cent or less for the storage period.

Ten-apple samples were made up when required in a set ratio basis according to tree crop size. Juice was prepared from these samples by quartering the apples in the entire sample and passing them through a Sweden Speed Juicer. The collected juice was sieved on a No. 35 wire-screen and then, after settling, was pipetted off in 25-ml. amounts for titration. Juice quantities were diluted 25 times with distilled water and then titrated with 0.1 N barium hydroxide, using phenolphthalein as indicator. The substantial dilution of the juice prevented interference of the end-point detection by the colouring matter inherent in apple juice.

The titrations with barium hydroxide were converted into milliequivalents of alkali required to neutralize 100 millilitres of juice. These figures in turn were fitted by the method of least squares to the function describing acid decline as outlined by Haynes (3):

$$\text{Ln } C = b - at, \qquad [1]$$

where "C" represents the acid concentration, "b" is a figure approximating the logarithm of the acid concentration at harvest, "a" is the rate of change of the logarithm of the acid concentration with time, and "t" describes time, usually in terms of days storage duration.

Computed "a" values from equation [1] were then tested graphically for linear relationship with temperature.

RESULTS

Data processed on the basis of equation [1] may be seen in Tables 1, 2 and 3. Titrations, deviations, and "a" values are all represented as natural logarithms. Five-place logarithmic tables were used throughout, though the number of digits shown was reduced for facility of reproduction and reading. The deviations tabulated are the differences between observed and calculated values of titrations, and the "a" values, or regression coefficients, indicate the rates of acid loss at various temperatures. The "a" values may be interpreted as the logarithms of the milliequivalents of acid consumed per day in 100 millilitres of juice. It may be observed that acid declines were calculated over the same concentration ranges for all temperatures for any one year. The insertion of the heavy lines in Tables 1, 2 and 3 distinguishes the pertinent data from those not used in the calculation of "a". The selection was based on observations made within a limited range of acid concentrations. These limits were defined by the amount of change in acid concentration in the apples held at 30°F. which kept over the indicated period in sound condition. The application of the same limits to the material from other temperatures assured the use of sound fruit.

Actually, all samples were composed of relatively sound apples. And so it may be both pertinent and interesting to examine the nature of the

TABLE 2.—TOTAL TITRATABLE ACIDS IN McINTOSH APPLES PICKED SEPT. 29, 1956, AND STORED AT INDICATED TEMPERATURES. SHOWN ALSO ARE THE RATES OF ACID LOSS, AND THE DEVIATIONS FROM THE LINES OF BEST FIT

30°F.			32°F.			35°F.			40°F.			55°F.		
Days in storage	ln titr'n m.e. baryta per 100 ml. juice	Deviation expressed as ln	Days in storage	ln titr'n m.e. baryta per 100 ml. juice	Deviation expressed as ln	Days in storage	ln titr'n m.e. baryta per 100 ml. juice	Deviation expressed as ln	Days in storage	ln titr'n m.e. baryta per 100 ml. juice	Deviation expressed as ln	Days in storage	ln titr'n m.e. baryta per 100 ml. juice	Deviation expressed as ln
11	2.68	−.020	11	2.73	+.039	11	2.65	−.015	11	2.65	−.019	11	2.57	+.035
11	2.68	−.016	26	2.69	+.046	11	2.65	−.022	24	2.62	+.032	11	2.57	+.039
27	2.64	−.002	48	2.54	−.003	26	2.60	−.002	46	2.46	+.007	25	2.30	−.069
47	2.56	−.014	69	2.42	−.045	46	2.52	+.017	67	2.35	+.018	41	2.13	−.060
68	2.53	+.032	69	2.43	−.035	67	2.47	+.058	79	2.20	−.058	48	2.09	−.017
89	2.43	+.010	89	2.35	−.043	88	2.33	+.021	102	2.12	+.002	48	2.12	+.006
103	2.40	+.025	104	2.29	−.038	103	2.21	−.030	122	2.02	+.019	59	2.05	+.066
123	2.30	+.002	123	2.27	+.017	122	2.12	−.041	152	1.79	−.030	66	1.84	−.065
152	2.22	+.022	152	2.19	+.043	152	2.03	+.013	170	1.77	+.060	79	1.94	+.183
171	2.14	+.004	172	2.08	+.018	170	1.92	−.017	206	1.49	−.056	102	1.84	+.398
207	1.96	−.043	207	1.93	−.005	207	1.82	+.059				121	1.66	+.387
												151	1.45	+.523
Rate of acid loss: "a" = 0.00355			"a" = 0.00388			"a" = 0.00463			"a" = 0.00599			"a" = 0.01148		

unused data shown below the heavy lines in Tables 1-3, which in some cases proved so divergent to the above and subsequent calculations that it was necessary to exclude them by definition.

For procedural reasons pertaining to other work, apples showing fungal wastage were rejected in sampling. This introduced a certain amount of selection of the material most resistant to rots, which would be more apparent at 50°-55°F. than at the lower temperatures. Fungal wastage at 50°-55°F. began to exceed 10 per cent in January, thus at least partially destroying sample identity. It may be observed that deviations indicated for the 50-55°F. unused data are largely positive which suggests that had these data been included in the calculations the acid descents would have been abnormally slowed.

In data pertaining to the storage temperatures of 35° and 40°F. no pattern of deviation in the unused data is apparent, largely perhaps because of insufficiency of titrations. It is interesting to note that Haynes (3) observed increased deviation in old or long-stored apples and also that the occurrence of breakdown precipitated the rate of acid loss. The authors have observed that core flush, a form of low temperature breakdown, often became apparent first in McIntosh stored at temperatures near 35°F., as opposed to McIntosh held at lower storage temperatures. There might then have existed here an inherent tendency to depress the acid concentration because of likely impending core flush in samples stored at 35°-40°F., suggested possibly in the data of Table 3.

In addition to the divergent and possible divergent influences of wastage disorders on the calculations it may be that the acid decline constant "a" is not altogether independent of acid concentration; and so again it appeared advisable and necessary to make comparisons of "a" values within the common limits of acid concentration changes experienced at 30°F. storage temperature as mentioned previously.

The seasonal constant "a" in the Haynes relation pertains to a particular storage condition, notably a particular temperature. When the "a" values in Tables 1, 2 and 3 were plotted against temperature on semi-logarithmic paper it was observed that a reasonably linear plot was obtained. It appeared that the rate of acid loss varied exponentially with the temperature of storage, and that we could write

$$a = D.e^{E\Delta T}. \qquad [2]$$

where "D" was a seasonal constant expressed as a logarithm, approximating a limiting condition of acid loss (viz., D = a, at freezing point), "E" was a seasonal value which partially controlled the response to temperature, and "ΔT" was the difference between the storage temperature and the freezing point of the apple expressed in degrees Fahrenheit. Then, substituting for "a", the Haynes' relation would read as follows:

$$\ln C = b - D.e^{E\Delta T}.t, \qquad [3].$$

Calculations and data pertaining to equation [2] are shown in Table 4. The E values are derived from the slope of the line of best fit and the D values come from extrapolation of the data through 1.5 Fahrenheit degrees

TABLE 3.—TOTAL TITRATABLE ACIDS IN McINTOSH APPLES PICKED SEPT. 19, 1957, AND STORED AT INDICATED TEMPERATURES. SHOWN ALSO ARE THE RATES OF ACID LOSS, AND THE DEVIATIONS FROM THE LINES OF BEST FIT

	30°F.		32°F.		35°F.		40°F.		50°F.	
Days in storage	ln titr'n m e. baryta per 100 ml. juice	Deviation expressed as ln	ln titr'n m.e. baryta per 100 ml. juice	Deviation expressed as ln	ln titr'n m.e. baryta per 100 ml. juice	Deviation expressed as ln	ln titr'n m.e. baryta per 100 ml. juice	Deviation expressed as ln	ln titr'n m.e. baryta per 100 ml. juice	Deviation expressed as ln
0	2.60	−.027	2.60	−.036	2.60	−.049	2.60	−.025	2.60	+.017
13									2.51	+.032
15	2.56	−.023	2.59	+.003	2.57	−.024	2.55	+.003		
32	2.56	+.034	2.52	−.013	2.56	+.035	2.48	+.009	2.29	−.043
39									2.31	+.035
49									2.17	−.031
54	2.49	+.028			2.54	+.093	2.35	−.013		
55			2.47	+.013					2.13	−.020
62									2.07	−.033
75	2.37	−.028	2.45	+.054	2.36	−.003	2.31	+.046	2.04	+.044
83									1.94	+.005
90	2.34	−.012			2.28	−.024	2.22	+.031	1.89	+.005
91			2.40	+.054						
95									1.86	+.015
105	2.30	−.000	2.28	−.014	2.24	−.016	2.09	−.026	1.77	+.002
112									1.76	+.046
123	2.28	+.036	2.19	−.049	2.18	−.006	2.00	−.026	1.67	+.043
146							1.92	+.006	1.56	+.110
147	2.18	+.011	2.16	−.003	2.09	−.007				
154									1.46	+.078
160			2.11	−.009			1.85	+.001		
161	2.13	+.001			1.94	−.098			1.40	+.073
169									1.34	+.073
176									1.31	+.094
181					1.94	−.022	1.68	−.067		
182	2.03	−.039			2.01	+.046			1.13	−.041
203	2.02	+.018	1.95	−.032	1.82	−.062	1.43	−.206		
204									1.13	+.129
Rate of acid loss:	"a" = 0.00308		"a" = 0.00321		"a" = 0.00377		"a" = 0.00484		"a" = 0.00777	

TABLE 4.—SEASONAL CONSTANTS GOVERNING THE RATE OF LOSS OF TOTAL TITRATABLE ACIDS IN MCINTOSH AND THE DATA ON WHICH THESE CONSTANTS ARE BASED

Storage temperature °F.	ln "a" + ln 1000	Deviation**	
30	1.42	−0.0083	Harvested—1955
32	1.50	−0.0110	E = 0.0419
35	1.66	+0.0213	D = 0.00392
40	1.85	+0.0043	t = 27*
50	2.26	−0.0064	
30	1.27	−0.0145	Harvested—1956
32	1.36	−0.0214	E = 0.0470
35	1.53	+0.0150	D = 0.00336
40	1.79	+0.0370	t = 32*
55	2.44	−0.0161	
30	1.12	+0.0264	Harvested—1957
32	1.17	−0.0257	E = 0.0475
35	1.33	−0.0074	D = 0.00279
40	1.58	+0.0046	t = 46*
50	2.05	+0.0021	

*Value of t required for significance at .01 level of probability = 5.84
**Difference between points listed in the preceding column and the calculated line of best fit

to an assumed freezing point of 28.5°F. The calculated "Student's" t values give an indication of the significance of the regression coefficients or E values. Computation of t values was based solely on the data in Table 4; the method used is described by Fisher (1, p. 143, sect. 26).

DISCUSSION

As an additional check on the linearity of the proposed semi-logarithmic plot described in equation [2], the data listed under Experiment 5 of Griffiths *et al.* (2) on Bramley's Seedling apples were processed in the same manner as our own after first removing the last two readings from the data listed under the lowest storage temperature (viz., 1°C.). These readings were removed because of their marked departure from the original trend. The t value for these data, indicating the significance of the regression coefficient, was 10.94 where 5.84 was required at the 0.01 level of significance. It was noted that these apples were extraordinarily immature, having been harvested in July at an average weight of 30 grams and that, typical of immature apples, there was a tendency for acidity to rise within the first few days after harvest. This undoubtedly influenced somewhat the initial rate calculations and consequently the final calculations. The logarithmic decline theory presupposes acidity to be on the downgrade at the time of harvest.

The E value calculated for the Bramley's Seedling apple was 0.0919 which on the basis of Fahrenheit degrees would be 0.0510. Data from Experiment 1 of Griffiths *et al.* (2), based on a more normal pick of Bramley's Seedling apple and held at seven storage temperatures (viz., 1°C. to 22.5°C.) were examined graphically. These data appeared to adhere adequately to the proposed temperature effect and suggested an E value of

0.027 which indicates that this variety of apple is more able to withstand high storage temperatures than our own McIntosh variety. Indeed the capacity of an apple to withstand high temperature, a very desirable characteristic at market, is often overlooked while at the same time comprehensive examination is made of low-temperature attributes. In this connection it should be observed that an E value of 0.0510 would multiply the basic rate of acid loss (D value) eight times, with a holding temperature of 68.5°F. ($\triangle T = 40°F.$); whereas under the same conditions an E value of 0.027 would only multiply the basic rate three times. In the calculation of "a" values from the data presented in Table 4 it is interesting to note that the rate of acid consumption of the 1956 McIntosh apples reaches the same proportion as those of 1955 at a holding temperature of 60°F.; at higher temperatures, of course, the 1956 rate surpasses the 1955 rate by an increasing margin. The "a" values at 60°F. were 0.015, 0.015, and 0.013 for 1955, 1956 and 1957 respectively.

Though no data are presented in support, it is interesting to speculate that possibly substantial differences in E values could be found in within-season comparisons as well, particularly if values for early and late harvested apples are compared.

As mentioned earlier, one of the applications of a relation associating acid changes with time is found in the estimation of storage life. Haynes (3) suggested that estimation was possible through the use of equation [1]. The experience of the authors, however, was that several months at storage temperatures would elapse before the acid concentration had changed sufficiently to adequately determine the particularized constant "a". If, however, representative material were subjected to two relatively high temperatures, acid changes would be accelerated and "a" values could be quickly determined. These two "a" values would then be translatable into storage conditions by solving equation [2] for constants E and D. For instance:

First taking logarithms and then solving simultaneous equations [2] yields

$$\ln a_1 - \ln a_2 = E(\Delta T_1 - \Delta T_2)$$

$$\text{or } E = \frac{\ln a_1 - \ln a_2}{\Delta T_1 - \Delta T_2}$$

Then substituting for E in the original equations [2], D may be determined from

$$D = \frac{e^{E \cdot \Delta T}}{a}$$

Finally applying determined values of D and E to equation [3], the time t to reach a specified level of acid concentration for a known storage temperature ($\Delta T + 28.5°F.$) is given by

$$t = \frac{\ln C}{b - D.e^{E \cdot \Delta T}}$$

CONCLUSION

The rates of decline of total titratable acids in McIntosh apples held at 30°-55°F. were found to vary exponentially with temperature and may be described in terms of acid concentration at any one time in the following manner:

$$\ln C = b - D.e^{E\Delta T}.t$$

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NOTE ON A PRECISION PLOT IRRIGATOR FOR USE IN IRRIGATION STUDIES[1]

Accuracy of irrigation research depends to a considerable extent upon the uniformity of application of a predetermined quantity of water to a plot area. Procedures most commonly used in the past were based on the use of either standard part circle sprinklers, or perforated irrigation pipe. Distribution of water from such equipment is seriously affected by air movement and even under ideal conditions uniformity of application of water is not satisfactory. The large plot area required and the equally large border effects using such equipment result in inefficient land use for research.

Precision plot irrigators have been designed and are in use at various centres (1, 3, 4, 5). Such equipment although accurate has generally been heavy and difficult for a single operator to move from plot to plot. Ease of movement preferably by one person is a very necessary feature of such equipment.

DESIGN OF EQUIPMENT

Irrigation experiments with horticultural crops require a yield area of approximately 400 square feet. This can be obtained by irrigating an area 25 feet in diameter and taking yield records on a plot 20 feet x 19 feet within this circular area. The design must permit uniform application of ½ to 1 inch of water per hour under a range of wind conditions. The equipment must be so designed that one man can readily move the irrigator and it should be a self-contained unit independent of either electric power or fixed water supply.

A preliminary irrigator was fitted with a modified water motor from an oscillating lawn sprinkler as the driving force to rotate the boom. Performance of this irrigator was adequate under calm air conditions but unsatisfactory when there was a wind. The model described herein was then built using the jet action of water from the spray nozzles to rotate the boom. A description of this irrigator follows.

Essentially the irrigator consists of a self-powered rotating boom supported on a tripod frame. (See Figure 1). Spray nozzles attached to the boom apply water and provide power to rotate the boom. The unit is simple in design and light in weight. It can readily be moved from plot to plot or from one area to another.

The tripod frame supporting the rotating boom is 4 feet in diameter with telescoping legs to provide a height adjustment of 24 to 48 inches. Legs are made of ⅛ x 2½ x 1½″ and ⅛″ x 2″ x 1″ aluminum channel with 4-inch base pads to provide support. Lengths of 2-inch aluminum pipe join the rotor standard to the tripod legs. These joints are welded.

The rotor standard is made of 2½-inch aluminum pipe, 7 inches long, fitted with two sets of ball-bearings. These support the rotor shaft and provide friction-free drive. The hollow rotor shaft extends through the standard and on the lower side is connected to a sealed coupling. A rotary

[1]Engineering Research Service Contribution No. 4, and Plant Research Institute Contribution No. 55, Research Branch, Canada Department of Agriculture, Ottawa, Ont.

pressure seal within this coupling prevents leakage with a minimum of friction. The coupling is secured to the tripod frame by means of a 'U' clamp and aluminum channel. Leading from the pressure coupling is a ½-inch copper pipe fitted with a hose coupling for connection to the source of water supply.

Extending through the upper side of the standard the rotor shaft is joined to a ¾-inch cross. Connected to the cross are the two booms and a vertical support pipe. Next to the cross on each boom is a union which allows for disconnection of the boom sections and which serves as a means for adjusting the angle of the nozzles. Support for the boom is by means of two wire cables fastened to each boom and to the vertical support pipe.

Nozzles on the booms are Spraying Systems Even-Spray Teejet with orifice No. 8002E. These are connected to the ¾-inch aluminum boom by Spraying Systems No. 7421 Split Eyelet Connectors. Each boom is approximately 12 feet long and has 18 nozzles. Spacing of the nozzles is calculated so as to give each an equal area of coverage. The following formula can be used to determine this spacing –

$$R_n = r_{(n-1)} + \frac{r_n - r_{(n-1)}}{2}$$

where $r_n = \sqrt{an/\pi}$

and $a = A/N$

A = Equivalent area in sq. ft. swept by boom in one rotation
N = Number of nozzles on one boom
a = Equivalent area in sq. ft. swept by one nozzle in one rotation
n = nozzle number
r_n = Radial distance of outer spray boundary, nozzle n
Rn = Radial distance of nozzle n.

The rate of water application by the irrigator can be found experimentally or it may be determined from the following formula –

$$i = \frac{2qN}{7.48} \times \frac{12}{A}$$

where i = Rate of water application in inches per hour
q = Capacity of one nozzle in gallons per hour (U.S.)
A = Area in sq. ft. covered by boom.

The speed of rotation of the boom is controlled chiefly by the nozzle angle; however, the operating pressure has some influence on its speed. With a nozzle angle of 25° with the vertical and an operating pressure of 35 pounds the speed of rotation is approximately 10 r.p.m. By varying the nozzle angle and operating pressure other speeds may be obtained.

With its simple design and few moving parts this irrigator should give lasting service with a minimum of care.

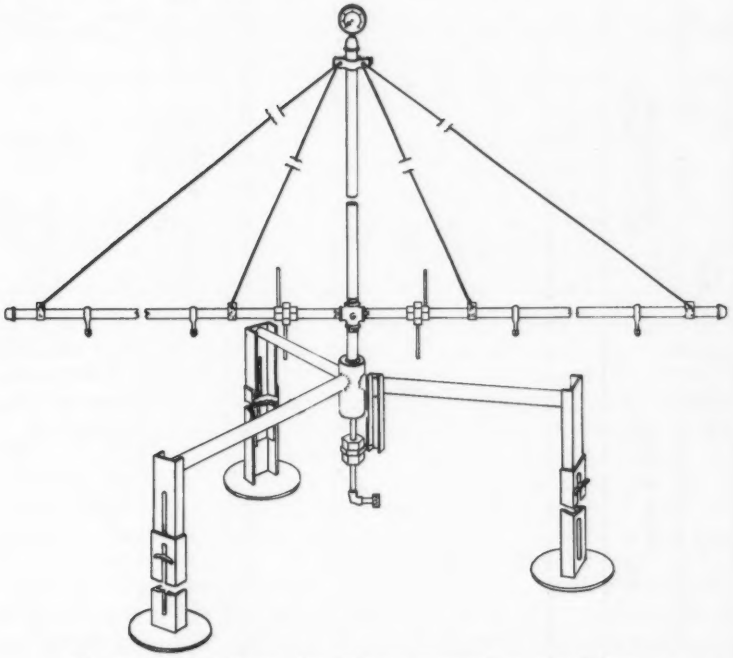

FIGURE 1. Twin boom plot irrigator mounted on tripod frame.

PERFORMANCE TESTS

Uniformity of distribution of water to the area was tested under a series of wind conditions on a 25-foot × 25-foot grid of open containers spaced at 1- and 2-foot intervals. In these tests the boom was operated 12 inches above the top of the containers. The coefficient of uniformity (2) was determined for each test and the delivery rate calculated when the irrigator operated at a pressure of 30 pounds per square inch at the boom. Results in Table 1 for the preliminary and the final model of the irrigator and for a perforated 4-inch irrigation pipe indicate the weakness of the latter under all conditions. This weakness applies to all equipment designed to throw water upwards and outwards. The model driven by the water motor, while a considerable improvement over the perforated pipe, was not satisfactory especially if the wind conditions were gusty or variable. This lack of uniformity was due to the inability of the motor to turn the boom at a constant speed against the wind resistance on the single boom. A further weakness was the relatively slow delivery rate from the single boom with 18 nozzles. The use of two balanced booms driven by nozzle pressure as in the final model resulted in very acceptable uniformity coefficients even in winds of 18-20 m.p.h. Such results are well above the acceptable level of uniformity.

Differences due to water pressure at the boom are relatively small and as indicated in Table 2 satisfactory uniformity of distribution is obtained

TABLE 1. — EFFECT OF AIR MOVEMENT ON RATE AND UNIFORMITY OF WATER APPLICATION WITH THREE PLOT IRRIGATORS

Air movement	Perforated pipe		Type of irrigator			
			Rotating boom driven by water motor[1]		Rotating boom driven by nozzles[2]	
	in./hr. Cu*		in./hr. Cu		in./hr. Cu	
Calm	1.01	73.4	.55	96.1	1.09	97.6
5-10 m.p.h.	1.23	68.7	.54	94.5	1.06	95.6
10 m.p.h. with gusts to 20	.87	48.9	.54	91.3	1.07	95.3
15 m.p.h. with gusts to 25			.46	78.6	1.03	94.5
18-20 m.p.h.					1.07	93.5

*Cu — uniformity coefficient
[1]18 nozzles with No. 80015E orifice
[2]18 nozzles with No. 8002E orifice and 18 nozzles with No. 80015E orifice

TABLE 2. — EFFECT OF OPERATING PRESSURE ON AVERAGE DELIVERY OF WATER AND UNIFORMITY COEFFICIENT OF IRRIGATOR OPERATED IN 5-10 M.P.H. WIND

Operating pressure	Water delivered per hour[1]	Uniformity coefficient
lb./sq. in.	in.	Cu
45	1.16	95.9
40	1.07	95.3
30	1.07	94.7
15–18	1.01	93.5

[1]18 nozzles with No. 80015E orifice and 18 nozzles with No. 8002E orifice

TABLE 3. — EFFECT OF 2 INCHES OF WATER APPLIED BY A PLOT IRRIGATOR ON THE SOIL MOISTURE CONTENT WITHIN AND BEYOND THE IRRIGATED AREA. IRRIGATOR APPLIES WATER 12½ FEET FROM CENTRE OF PLOT

Soil depth (in.)	% Moisture before irrigation	Per cent soil moisture after 2 inches of irrigation							
		Distance from centre of plot, feet							
		2	6	10	12	13	14	15	16
0–4	17.1	29.5	33.2	29.1	29.5	27.1	22.0	17.1	17.3
4–8	14.9	28.4	24.0	23.3	24.2	17.8	18.9	15.0	15.7
8–12	10.1	18.1	21.4	17.5	17.5	17.4	17.3	13.3	9.5

over a range of 15 to 45 pounds per square inch. Since trials showed up differences between manufacturer's ratings and actual delivery rates it is recommended that units be calibrated under field conditions. For best results an operating pressure between 30 and 40 pounds is recommended.

An important consideration in plot irrigation work is the border effect likely to result from water application. A test was made on the final model of the irrigator in which 2 inches of water were applied to the soil and the percentage of soil moisture determined at three depths 1 hour after irrigation. The results of this study, shown in Table 3, indicate that water applied by the irrigator cannot be shown in the soil more than 3 feet from the edge of the irrigated area even on the soil surface. This permits a close spacing of plots and with this equipment adjoining plots 25 feet x 25 feet have been shown to be satisfactory when 2½ feet are left as border around the plot. This is approximately one-quarter the size of the plot required using the perforated pipe method of water application.

Field trials have shown that the equipment can be readily moved from plot to plot and if necessary from area to area using a small truck. By using two irrigators on a single pump it has been possible to irrigate up to 12 plots per day. Depending on the size of pumping unit, virtually any number of sprinklers can be operated at the same time. For example, a 35 g.p.m. centrifugal water pump powered by a 1½ h.p. gasoline engine could supply sufficient water to handle five irrigators simultaneously.

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Erratum:

In the paper by Kilcher and Lawrence, entitled "Quality of intermediate wheatgrass seed, with and without hulls", published in the July, 1960, (Vol. 40, No. 3) issue of the "*Canadian Journal of Plant Science*", on page 486, line 17, the correlation coefficient figure shown as "±.529" should have read "+.529".

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